The biological effects of *Lupinus polyphyllus* on soil nitrogen in acid high country soils

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Many high country soils in South Island have low soil pH and possibly high exchangeable aluminium (Al) concentrations, which limit establishment and persistence of pasture legumes for nitrogen (N) contribution. *Lupinus polyphyllus* (common name 'Russell lupin', also known as perennial lupin) is a species of flowering plants in the legume family, Fabaceae. Perennial lupin is able to grow in acidic soil (pH < 5.5) with high levels of exchangeable Al (Al>3ppm, 0.01 $CaCl_2$) toxic to most other legumes, and low soil P (Olsen P <10). The growth ability of perennial lupin indicates that it is better adapted to low fertility environment than other conventional pasture legumes. The high tolerance of lupin to low fertility and acid soil shows that it occupies a unique edaphic niche among forage legumes. As such, this species may be suitable to develop poor quality soils on south island high country farms in South Island, New Zealand.

This study focused on the biological effects of perennial lupin stands of varying ages on soil N and carbon (C) concentrations in acid high country soils. There were two experiments in this study: a field experiment and a glasshouse experiment.

For the field experiment, soils were collected and analysed for total soil N, total soil C, soil mineral N, mineralizable N, soil pH, soil Olsen P and sulphate sulphur (S) from eight lupin stands of different ages and adjacent established pasture sites. There are eight sites across four farms: Sawdon Station, Glenmore Station, Omarama Station, Dasher Station and one from Lincoln University Campus. To quantify soil labile N status more directly, the glasshouse experiment also was conducted in soil from five sites from the field study. Annual ryegrass (*Lolium multilforum*) was grown in soil collected from established lupin stands and grass-dominant pasture, and exhaustively harvested to extract the labile soil N. Ryegrass dry matter yield and shoot N were analysed for calculating soil N status. Shoot N was analysed as a measure of labile soil N, in an effort to quantify the effects of perennial lupin biological

N inputs to this suite of field soils. Adjacent long-term pasture soils were also included as comparative baseline controls.

In the field experiment, perennial lupin significantly increased total soil N and soil mineralizable N in the plant rooting zone of high country soils and lupin stand soils showed higher soil N status than pasture soils, resulting from N fixation. The highest value of soil mineralizable N among eight sites occurred in Glenmore Station and Lake Tekapo (site 2) soils with 213.8 kg/ha. Soil N status also declined with increasing soil depth in both lupin soils and pasture soils caused by plant residue accumulation in the topsoil. Soil N level generally increased with increasing lupin stand age.

The glasshouse experiment showed a similar trend as the field experiment, that lupin soils exhibited higher soil N status than pasture soils at each site. Higher soil mineralizable N lead to higher ryegrass dry matter yield and higher N uptake. Soil N level also decreased with increasing soil profile depth in both lupin stand soils and pasture soils. Ryegrass dry matter yield and N uptake by ryegrass generally increased with lupin stand age. The significant differences between each site in both experiments were caused by annual rainfall and soil conditions.

This study provides strong evidence that lupin substantially increases soil total and labile soil N, which increases with lupin stand age.

Keywords: *Lupinus polyphyllus*, nitrogen, high country soil, pasture, stand age, aluminium, acid soil

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Chapter 1

Introduction

The productivity of high country pastures in South Island, New Zealand, is typified by a short, often soil moisture-limited growing season. Alternative pasture species have been suggested to improve dryland pasture production (Brown *et al*., 2009; McGowan *et al*., 2003), and moreover, the deep-rooting nature of lucerne (*Medicago sativa* L.) has highlighted this species in dryland environments (Brown *et al.*, 2009; Thomas, 2003). However, lucerne is highly intolerant of acid soil conditions and related aluminium (Al) toxicity (Rechcigl *et al*., 1988; Su *et al*., 1996).

Many South Island high country soils have low soil pH and possibly high exchangeable Al concentrations. Soil acidity (low soil pH coupled with toxic concentrations of exchangeable Al) and low available phosphorus (P) and sulphur (S) may also limit establishment and maintenance of legumes (Haynes & Williams, 1993; Moir *et al*., 2000). To neutralise increased soil acidity, lime ($CaCO_3$) must be applied, and where this cannot be done economically, soils may be too acidic for legumes and productivity declines sharply (Edmeades *et al*., 1983; Lanyon & Griffith, 1988). Often the cost of lime or its application is uneconomic in extensive high country regions. In many cases the rate of penetration of the effects of surface-applied lime, and subsequent pasture response is unknown. Incorporation of lime may be possible on flatter land but the risk of wind erosion means direct drilling is the preferred establishment technique. The relationship between pasture production and soil pH is well established on some high country soils and the critical concentrations of exchangeable Al (Haynes & Williams, 1993) and in the same case, the relative Al tolerance of some forage legumes have also been examined (Edmeades *et al*., 1991; Moir *et al*., 2016; Wheeler *et al*., 1992). However, studies on alternative legumes that are tolerant of low soil pH and high Al in South Island high country soils are scarce (Moir *et al*., 2016).

Lupinus polyphyllus (common name 'Russell lupin'), but also known as perennial lupin, is a herbaceous perennial legume species, which has the potential to improve soil N and biological N cycling (Scott, 1989). Perennial lupin is adapted to live in low fertility soils, particularly acid soils, shown by the work of Dr David Scott in the high country surrounding Mt John in Tekapo, New Zealand. Trial sites which were abundant in lupin and received low to moderate fertiliser inputs (0-100 kg superphosphate/ha/yr) produced yields of 5-7 t of dry matter (DM)/ha/yr (Scott, 2000). However, plant yields were determined by the specific environmental conditions at a site including soil fertility, rainfall and management practices. It has been suggested that lupin has the potential to replace traditionally used clover species in hill country areas, where the soil fertility is marginal and

the application of fertilisers to improve this is uneconomic (Nordmeyer & Davis, 1977; Scott, 1989; Wangdi *et al*., 1990). In low P conditions, lupin out yield clovers (Davis, 1981b). This is because lupin form mycorrhizal associations in the soil to increase the surface area for absorption of P and utilises P that is unavailable to most other plants. (Borie *et al.*, 1990; Davis, 1981a). Perennial lupin has a large root system which in loose textured soils improves both the soil structure and reduces the rate of erosion, enhancing soil conservation (Rowland *et al*., 1986).

Perennial lupin may have a tolerance to high soil Al levels associated with acidic soils, where other legume species will not grow (Nordmeyer & Davis, 1977; Scott, 1989). This species is assumed to biologically fix atmospheric N into the soil / plant / animal ecosystem, which significantly benefits companion species including grasses. Lupin is dormant in the Mackenzie basin area during the winter, but grows well in the springtime (Taylor, 2013).

Although recent research by Black *et al*. (2015) has indicated moderate productivity of perennial lupin in acid high country soils, the effects of lupin on soil N and biological N and C in soil has not yet been studied. As such, the quantity of biological N inputs and effects on critical soil N and C biochemistry from lupin is unknown and undocumented in scientific literature. This research aims to examine and quantify changes in soil biological N in acid soils where perennial lupin has been grown for several years in the South Island. Sites will also be contrasted regarding how many years the lupin has been present.

The objective of this study is to determine the effects of perennial lupin stands of varying ages on N concentrations in acid high country soils in South Island, New Zealand. There are two hypothesises in this study. The first hypothesis being tested is that perennial lupin increases soil total and mineralizable N in the plant rooting zone of acid high country soils. Secondly, the extent of soil N accumulation in soils growing lupin is dependent on the age of the lupin stand.

Chapter 2

Literature Review

This literature review introduces the importance of N for plant and the N cycle between the non-living systems and living systems. Soil N accumulation and the N fixation by conventional pasture legumes are the main focus of this review. The review aims to current knowledge of perennial lupin and its advantages of perennial lupin among traditional forage legumes in high country soils.

2.1 High country soils

Most high country soils in South Island, New Zealand, are brown soils, pallic soils and semiarid soils with poor soil fertility, such as low plant available N, P and S, which is caused by weathering processes such as wind and water erosion, limited plant growth and pasture productivity (Langer, 1990). Most high country soils are also acidic with a low soil pH of 5.5 and occur in a high leached environment, which could increase soil acidity (McLaren & Cameron, 1996).

2.1.1 Low soil pH

Many high country soils in the South Island have low soil pH and possibly high exchangeable Al concentrations (Moir & Moot, 2010). Soil pH and concentrations of exchangeable Al in soil are highly inversely related to each other. Low soil pH limits the plant root growth and nutrients uptake, which limits establishment and persistence of pasture legumes and, therefore, their ability to fix atmospheric N. Edmeades *et al*. (1984) suggested that 5.8 to 6.0 was the ideal pH for NZ grassland soil. To fix the low soil pH and high concentration of exchangeable Al in high country soil, the most efficient way is applying a high rate of lime at on the soil surface (Moir & Moot, 2010). Remediation of acid soil by lime changes the nutrients form and high plant available nutrients for plants utilise (McLaren & Cameron, 1996). However, the cost of lime application in high country soils is uneconomic and to have a significant effect on subsoil pH and exchangeable Al, lime application is necassary long-term, taking 5 to 8 years in the 7.5 to 15 cm soil depth (Moir & Moot, 2010).

2.1.2 High exchangeable Al concentration

The harsh environment of high country soil is also caused by high exchangeable Al concentrations. Aluminium is toxic for many plants when the soil exchangeable Al concentration is higher than 2-3 ppm with a low soil pH (<5.5) (Påhlsson, 1990). Although, the tolerance of Al toxicity varies by plant species, most conventional pasture legumes have low tolerance of Al toxicity (Schroth *et al*., 2003). Even in the same plant, Al toxicity varies by plant ages, with seedlings being more susceptible than older plants (Thawornwong & Van Diest, 1974). It is hard to identify the symptoms of Al toxicity,

while it can be observed in plant roots as thick, shorter roots with less branching (Bennet & Breen, 1991; Rout *et al*., 2001). The symptoms of Al toxicity in plant roots result from root damage, as Al toxicity limits root growth and, therefore, reduces nutrient and water acquirement (Rout *et al*., 2001).

Due to the low soil pH and high exchangeable Al concentration condition of high country soils, the legume species that have high Al toxicity tolerance and survive with low soil pH have the advantage among conventional pasture legumes in increasing pasture production (Moir & Moot, 2010).

2.2 The importance of N for plants

Nitrogen plays an important role in plant nutrition. Low concentrations of soil N often limit pasture and crop yields. Although N is one of the most abundant elements on earth, N deficiency is the most common nutritional problem affecting plants. This is because most N is either atmospheric N or as organic N in soils. Nitrogen is also necessary for many functions in plants. It is a primary constituent of the basic amino acids, which are the important components of proteins. It is also a constituent of chlorophyll and high-energy compounds such as some plant hormones. Nitrate is essential for plant nutrition, which can help tissue development and build the immune system. It is also beneficial to plants for reproductive development and seed production. Nitrate is the most common form of inorganic N, which is the most usable form to plants. As N is most often the limiting nutrient in plant growth, N fertilisers can be used to overcome this limitation and increase pasture and crop yields.

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Figure 2.1: The nitrogen cycle in the soil, the nitrogen forms and the processes of create and recycle, including inputs and losses from the system (Cameron, 1992).

2.3 Nitrogen cycle in the soil / plant / animal system

The main reservoir of N is the atmosphere, because 78% of atmosphere is N (N_2). The efficient cycling of N is necessary to plants to get nutrition. The cycling is between the non-living system and living system. Figure 2.1 above shows the N cycle in an agricultural system.

2.3.1 Gains

The three main ways to gain the N to the agricultural soil. Nitrogen fertilisers are important to plants, which comes into used by human. Nitrogen fixation by legumes is also a way for agricultural system to gain the N. Additionally, N from animal manure is another resource of N gaining.

2.3.2 Transformations

There are four main processes of N cycle:

a) Nitrogen (N_2) fixation

Atmospheric N_2 cannot be used by plants directly. It must be fixed to the N from gaseous to solid form. Most of N is fixed by certain bacteria, diazotrophs, such as rhizobia. The bacteria can use nitrogenase enzyme to combine the N_2 from the atmosphere and change them to ammonium (NH_4^+). This process is the way to convert inorganic N into organic N compounds. For example, free-living soil bacteria, rhizobia, which in legume root nodules forms a symbiosis with legumes can fix N_2 to provide the N to legumes. At the same time, the legumes provide carbohydrates to rhizobia. They have a mutualistic relationship. On the other hand, some free-living N fixation micro-organisms are not carried out by higher plants directly. Some other bacteria are also able to fix N. In addition to biological fixation, N fixation process can also be carried out during lightning storms. The amount of N fixation can be influenced by many factors, such as environmental conditions and different plants.

b) Ammonification

In this process, the organic N from dead parts of plants and animals or animal excretion is decomposed. Following the breakdown of complex proteins into amino acids, the amino acids are transformed into ammonia (NH_3) by soil organisms. The process is carried out by microorganisms in the soil, like bacteria or fungi.

The soil pH can affect the ammonification, and under the high pH, the NH_3 gas production is favoured. The relative equation is below from Haynes and Sherlock (1986) is:

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \qquad (1)$$

The hydrolysis process of urea can produce the ammonium carbonate which may dissociate in turn to produce ammonium, ammonia and hydroxide ions. The equation of this process is:

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3 \rightarrow NH_4^+ + NH_3\uparrow + CO_2 + OH^- \qquad (2)$$

c) Nitrification

Nitrification is an oxidation reaction carried out by certain bacteria in the soil. Ammonium and nitrate (NO_3^-) in soil, which from soil organic matter can be converted into NO_3^- with the O_2 in the soil. There are two major steps in this process. In the primary stage of nitrification, NH_4^+ is transformed to nitrite (NO_2^-). This step is carried out by NH_3-oxidising bacteria (AOB), such as Nitrosospira and Nitrosomonas, and occurs by the ammonia monooxygenase (AMO) enzyme associated with the bacteria (Ferguson *et al*., 2007). The second step is oxidising NO_2^- into NO_3^-. And this further oxidisation is carried out by one genus of bacterias called Nitrobacter. Due to the second step takes place very rapidly, NO_2^- rarely accumulates in the soil.

In nitrification, the recreations of two steps oxidation (Cameron *et al*., 2013) are:

$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$ (3)

$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy}$ (4)

d) Denitrification

Denitrification is a way to return NO_3^- back to N_2 and others type of N (NO, NO_2, N_2O). It converts organic N into inorganic N, which is also carried out by microorganisms in the soil. Denitrification is performed by specific species, and occurs through two mechanisms: biological denitrification and chemical denitrification. The biological denitrification happens in poorly drained soil and anaerobic conditions. In this part, anaerobic bacteria use NO_3^- as an electron acceptor, in place of O_2, during respiration. The productions of denitrification are gaseous N_2 and gas N oxides. These types of N are unavailable to plants, which can be lost from soil to the atmosphere. Although denitrification occurs in waterlogged soils generally, it can also occur in imperfectly drained soil. It depends on soil redox potential drops. In chemical denitrification process, the N losses in gaseous form have had NH_4^+ fertilisers applied. High concentration of NH_4^+ limits the activity of Nitrobacteria and result in NO_2^- accumulation, may cause loss of N_2 independent of microbial activity. The certain enzymes participate in both denitrifications.

2.3.3 Losses

Losses, animal uptake, plant uptake, volatilization, denitrification and leaching are the important ways of losing mineral N in agricultural and horticultural systems.

a) Ammonia volatilization

In NH_3 volatilization, gaseous NH_3 lost from soil surface to the atmosphere. The volatilization of NH_3 happens through dry deposition or wet deposition generally. The loss of gaseous NH_3 not only causes the loss of N from soil but also has been a threat to environment. The potential risks from NH_3 volatilization from urea fertiliser has impacts on 0-65% of the N applied. The NH_3 volatilization is usually highest when the temperature and soil pH is high.

b) Leaching

The different rates also depend on different soil and climatic conditions. Nitrate leaching is another loss of N which losses N fertilisers from soil into water. It respects a threat to wider environment and human health. The pollution of NO_3^- leaching in water can contribute the drinking water supply severely, including a risk of methemoglobinemia in babies and has been linked to some cancer and

heart disease. The nitrate which may be leached into lakes or rivers may cause eutrophication, which may result in algae blooms and the O_2 deprive condition can cause the death of fish.

2.4 Soil N accumulation

Soil N accumulation is affected by many factors, including climate, age of the pasture, fertiliser application, plant species, pasture slope, soil management and so on. Symbiotic N fixation is a process of energy demanding. As previously stated, legumes can fix N from atmosphere to the soil. If N supplement is adequate and available from the soil, legumes obtain less N requirement from the atmosphere. At the same time, high levels of mineral N inhibit legume nodule formation by inhibiting the numbers of infective sites, successful infections on the primary roots, and nodule growth and functionality (McLaren & Cameron, 1996). Allos and Bartholomew (1959) also indicated when the quantity of N application exceeded the requirement for maximum growth, the increasing inorganic N rates decreased numbers and size of nodules which applied at regular weekly intervals and reduced N fixation. Increasing amount of fertiliser N or urine N increases the amount of N uptake by legumes. When N application exceeds the necessary rate of growth, N uptake partly replaces N_2 fixation from plant-available N. Allos and Bartholomew (1959) found that the increasing N application decreased the rate of total N fixation in several legume species (*Glycine max, M. sativa, Melilotus spp., Trifolium repens and Lotus corniculatus*). Ledgard and Steele (1992) also reported the natural feedback mechanism between pasture legume N_2 fixation and soil inorganic N and competition from associated grasses. Under low soil N pasture, legume dominated and drive most N from N_2 fixation. However, under high soil N pasture, grasses dominate and have a competitive advantage than legumes.

Melville and Seaes (1953) reported that the climate in New Zealand provides a relatively better environment for plant growth in mixture grass and legume (clover) pastures through the year. In addition, a large amount of N can be symbiotically fixed under the pastures in the mixture pasture with the increase of soil organic matter. Jackman (1964) reported that the relationship between the age of permanent pasture and the N content in the soil from the last ploughing. Jackman (1964) measured 10 soils in three levels (0-3, 3-6, and 6-12 inches layers) located in ten different places in New Zealand (Oropi, Taupo, Tiniroto, Tirau, New Plymouth, Egmont, Hamilton, Matapiro, Tokomaru, and Waiotu). Figure 2.2 shows the content of N in different mentioned pastures. Excluding Taupo, soils in the other nine places had been prepared as a normal pasture, ploughed, cultivated, cropped, and then reploughed and cultivated usually. In Taupo, the soil was not farmed before sowing to permanent pastures, and the seedbed had been prepared only by ploughing or burnt-over. Each pasture has been applied superphosphate as a fertiliser with different rates.

Generally, the contents of N increased markedly in top 7.5cm and slightly increased or no significant change under 3 inches to 12 inches (Figure 2.2). However, in Tirau and Tokomaru soils, soil N content increased in top 3 inches and declined in 3 to 6 inches. In addition, the little change of N content in 6-12 inches had been partially balanced by the changes above 6 inches. The value of N content decreased by the depth of soil and showed obvious differences in three levels. The ten different soils were ranked according to the contents of soil N measured. New Plymouth showed the highest values in the soil N content in the top-soil, while the values of N content in Tokomaru soil described the lowest values in those ten different soils.

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Figure 2.2: The relationship determined between the age of permanent pasture, and the content of N in the soil from the last ploughing in ten places in New Zealand (Jackman, 1964).

The values from Figure 2.2 indicated the total soil N content increased in a long-term natural pasture which caused by continuous fertility input. Jenny (1941) explained the reason for these results and shown the changing patterns in soil organic matter according to measure the relative organic matter input and output rate in the pasture. Jenny (1941) indicated that the main input of plant residues is close to the soil surface in a long-term pasture. Therefore, the organic matter content in soil increased by the increasing time after ploughing in the top of ploughing layer. The decrease in the bottom of ploughing layer caused by a higher initial content and the greater distance from the soil surface where the main organic matter input was sourced from.

Moir *et al*. (1997) reported the effect of fertiliser history on both plant-available nutrient and total nutrient accumulation in New Zealand hill country legume-based pasture in fourteen trial sites. They illustrated that continuous fertiliser application increased total soil fertility by increasing of soil N accumulation. In their experiment, they applied single superphosphate (SSP) on the pasture with 3 different levels (no fertiliser; low; medium with 0, 125 kg/ha SSP or high 250+ kg/ha SSP) for 15-20 years as a long-term fertiliser application. Figure 2.3 shows the relationship between total soil P and total soil N contents. It is apparent that soil total N was poorly related to total soil P. Figure 2.4 shows the relationships between Olsen P and soil mineralizable and mineral N: total N ration. However, soil mineral N levels ranged from 110 to 300 kg N/ha, and there is a linear relationship between soil mineralizable N and soil Olsen P (R^2=0.80) (Figure 2.4). The large difference between the two relationships was affected by the increase of soil P fertility, which changed nature of soil organic N. The content of soil organic N was changed to be readily mineralizable which could be used by plants. Continuous fertiliser application increased soil fertility by increasing the growth of legume. At the same time, legumes fixed the N and the residue returned the N to the soil which increased soil total N content.

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Figure 2.3: The relationship between total soil P (ug P/g) and total soil N content (ug N/g), each point represent the total soil P and total soil N content if a trial site in the Wairarapa region (central and southern east-coast of the North Island, New Zealand) (Moir *et al*., 1997).

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Figure 2.4: The relationship between Olsen P and soil mineralizable N (solid line, standard text), and mineral N: total N ratio (dashed line, bold italics), each number represent a trial site soil in experiment (Moir *et al*., 1997).

Watson *et al*. (2007) reported the influences of soil total N accumulation in the grazed grassland affected by different rates of N inputs. They set six different levels of N input (wet deposition + fertiliser N applied) from 0 to 500 kg N/ha/year for 10 years' long-term application and calculated the accumulation of soil N by the N input minus N output (drain flow + animal product). They measured the soil N content in 3 depth levels (0-5, 5-10 and 10-15 cm). Table 2.1 shows the total N accumulation in different levels of soil affected by different fertiliser N application rates. In Table 2.1, the total N accumulation increased by increasingly fertiliser N applied. The accumulation was significant in top 5 cm of soil that over 80% of total N accumulation occurred at this level and smaller N accumulation at 5-10 cm of soil. However, a marked decrease happened at 10-15 cm depth. The decrease in the bottom level might be caused by the longer distance between the 10-15 cm and the organic residual on the soil surface which was explained in previous studies by Jenny (1941) and (Melville & Seaes, 1953).

Table 2.1: Total N accumulation in soil. Values within parenthesis represent the standard errer for the extrapolated zero N applied (Watson *et al*., 2007).

	Soil depth (cm)			
Fertiliser N applied (kg N/ha/yr)	0-5	5-10	10-15	Total (0-15cm)
100	82	34	-14	102
200	90	36	-12	114
300	98	39	-10	127
400	106	41	-8	139
500	114	43	-6	152
Standard Error	3.3	4.2	3.7	6.5
Zero N applied	74(4.4)	32(5.5)	-17(4.9)	90(8.6)

2.5 N fixation

2.5.1 Clovers

Clovers are the small annual, biennial, or perennial herbaceous plants and a type of legume, which means it can also fix N_2 to the soil to increase soil N, reducing the need for synthetic N fertiliser. It has been known that several species of clover are extensively cultivated as fodder plants, such as white clover (*Trifolium repens* L.) and red clover (*T. pratense* L.) which are most widely cultivated with ryegrass (*Lolium perenne* L.) as fodder on the pasture land and are palatable to and nutritious for livestock (Caradus *et al*., 1996; Harris *et al*., 1997). In New Zealand, most pastures have less than 20% clover content, which is lower than the requirement of maximum N fixation and animal performance (Harris *et al*., 1997; Ledgard & Steele, 1992). Some clovers can be used over the range of environmental conditions (Caradus *et al*., 1996). Many estimates of N fixed by white clover have been reported in New Zealand, were much higher in white clover than those reported for perennial lupin and lucerne (Hoglund *et al*., 1979; Jackman, 1964; Sears & Evans, 1953; Sears *et al*., 1965).

Like other legumes, white clover can utilise two types of N sources: atmospheric N_2 and soil mineral N. Mineral N is used preferentially if enough plant-available N in soil. However, fixation and assimilation of mineral N usually proceed concurrently when the soil without a large content of mineral N (Hoglund & Brock, 1987). Ball *et al*. (1978) reported that the increasing application of N fertiliser on pasture land led to a decrease in clover content, due to the increased competition from grasses.

Crush (1987) reported that the potential N fixation rate from white clover was in the range of 600-700 kg N/ha/year. Sears *et al*. (1965) also reported the 650 kg N/ha/year fixed by white clover in Palmerston North. However, the presence of mineral N and many factors those limit the growth of white clover result in higher clover N fixation rates by white clover. Nitrogen fixation rate can be affected by many factors, such as soil conditions, grass competition, fertiliser application rate and enough appropriate *Rhizobium* strains. And annual N fixation rates from white clover in a grazed pasture are extremely variable, ranging from 17 kg N/ha/year in no fertiliser application, unimproved hill pastures (Grant & Lambert, 1979) to 380 kg N/ha/year in intensively managed pastures (Rumball, 1979). Hoglund and Brock (1987) also indicated that N fixation and growth of white clover were dependent on seasonal soil temperature, soil moisture and soil management. Hoglund *et al.* (1979) indicated the N fixation rate varied widely among nine grazing pasture in New Zealand, caused by the soil conditions, climate and the different competition from various grass species. Due to the competition with its associated pasture species, white clover takes up the N directly from the soil to meet the part of N requirement. This means that in a grazed pasture, increasing clover yield does not mean an increase in N fixation. And without mineral N in the soil, there is a relationship between N

fixation and white clover growth directly that the increasing mineral N reduced N fixation (Allos & Bartholomew, 1959; Moustafa *et al.*, 1969).

Table 2.2: Effect of N fertilisation and stocking rate on total production, clover production, N_2 Fixation and grass N uptake averaged over 5 years. Nominal rates of N fertiliser were 0, 200 and 400kg N/ha/yr and the subscripts L and H refer to stocking rates of 3.3 and 4.4 cows/ha, respectively. SED derived from ANOVA (n=4; data averaged for duplicate plots within paddocks and across all years); ns not significant, * *P*<0.001. [a]: 2Proportion of total clover N from N_2 fixation. [b]: Based on 1.7 x fixed N in herbage (Ledgard *et al.*, 2001).**

	0 N_L	200 N_L	400 N_L	400N_H	SED
Pasture production (kg DM /ha/yr)	16380	18450	20580	20480	527***
Clover content (%)	15.2	10.7	4.9	7.0	1.3***
Clover production (kg DM /ha/yr)	2494	1964	997	1425	265***
Clover %N	4.60	4.68	4.70	4.66	0.05ns
Total clover N (kg DM /ha/yr)	116	93	48	66	12***
P_N[a] (%)	76.7	63.7	48.3	42.9	3.0***
Fixed N in herbage (kg DM /ha/yr)	90.7	58.0	22.8	31.4	9.0***
Total fixed N[b] (kg DM /ha/yr)	154	99	39	53	
Grass N (kg DM /ha/yr)	442	596	753	699	27***

Many studies showed the similar changes or trends of N fixation by clover drive by the N fertiliser application rate, stocking rate and seasonal climate conditions. For example, Ledgard *et al*. (2001) reported the effects of N fertiliser and stocking rate on production and N_2 fixation by white clover grown with perennial ryegrass. The experiment was determined over five years on a farm near Hamilton, New Zealand. Three urea application rates (0, 200 and 400 kg N/ha/year) were used on three plots carried 3.3 dairy cows/ha. Another plot received 400 kg N/ha/year with 4.4 cows/ha.

Table 2.2 shows the effect of N fertiliser application rates and stocking rates on total production, clover production, N_2 fixation and grass N uptake averaged over five years. In Table 2.2, most of the values (total clover N, clover proportion, total clover herbage accumulation, the proportion of total clover N derived from N_2 fixation and total N fixation) declined from 0 N application to 400 kg N/ha/year urea application in lower stocking rate. In addition, those decreases were greater in lower stocking rate than in higher stocking rate with 400 kg N/ha/year urea application. Conversely,

fertiliser N application increased total pasture production and grass N content, with more significant increase rates in lower stocking rates than higher stocking rates. There was no significant effect on N fertiliser applied levels on N concentration in clover. Thus, annual clover accumulation reflected differences in clover herbage accumulation.

Table 2.3: Effect of N fertilisation and stocking rate on seasonal clover production, N_2 Fixation. Data are means for 5 years. Nominal rates of N fertiliser were 0, 200 and 400kg N/ha/yr and the subscripts L and H refer to stocking rates of 3.3 and 4.4 cows/ha, respectively. SED derived from ANOVA (n=4; data averaged for duplicate plots within paddocks and across all years); ns not significant, * *P*<0.001 (Ledgard *et al*., 2001).**

	0 N_L	200 N_L	400 N_L	400N_H	SED
Clover production (kg DM/ha)					
Spring	913	565	383	530	83***
Summer	935	577	259	436	106***
Autumn	472	620	230	343	70***
Winter	278	193	109	160	35***
Clover total N concentration (%)					
Spring	4.76	4.84	4.81	4.77	0.07ns
Summer	4.45	4.50	4.56	4.41	0.08ns
Autumn	4.72	4.85	4.78	4.93	0.10ns
Winter	4.66	4.65	4.54	4.56	0.12ns
Proportion of clover N fixed (%)					
Spring	77.8	64.6	48.7	45.1	4.3***
Summer	80.0	67.6	51.5	45.0	5.2***
Autumn	78.2	59.0	46.4	37.7	4.2***
Winter	68.3	51.3	39.3	30.8	5.0***
Fixed N in herbage (kg N/ha)					
Spring	33.9	17.8	9.1	11.8	2.9***
Summer	33.7	17.8	6.1	11.2	4.0***
Autumn	17.5	17.8	5.2	6.9	2.5***
Winter	9.0	4.6	1.8	2.3	1.0***

Table 2.3 shows the effect of N on clover herbage accumulation varied seasonally. In 200 N treatment, seasonal differences were most significant with a decrease in clover herbage accumulation during spring and summer, while clover herbage accumulation in autumn was greater in 200 N than in 0 N during 5 years. The results of their experiment showed the marked effect on N_2 fixation by the N fertiliser application rate, estimated the decline in annual N_2 fixation about 0.27 kg N fixed by each kg N applied. The results can be explained by the decrease of clover production driven by the competition with other species on pasture. The addition effect from fertiliser application rate was due to the replacement of fertiliser N for N_2 fixation. The lower proportion of total clover N from N_2 fixation caused by intensive winter grazing management. The higher grazing rate about 400 cows/ha on small areas with the daily shift to new pasture led to the treading damage on wet soils (Ledgard *et al*., 1996) which reduced the clover function. Gibson (1977) reported the

faster clover growth and more N absorption when dependent on uptake of inorganic N than when reliant on N_2 fixation under low soil temperature. Additionally, during this period, the low soil temperature reduced nodule function as well. In addition, the same values of N_2 fixation resulted from 200 N treatment and 0 N treatment in autumn, while higher clover production under 200 N treatment. The differences of clover growth by two treatments were attributed to more severe grazing during summer in the 0 N treatment, which led to the damage on clover stolon and higher surface soil temperatures.

2.5.2 Lucerne

Lucerne (*Medicago sativa* L.) is a perennial flowering forage legume. It has high water use efficiency and the high tolerance of drier environments, due to the deep-tap roots. Lucerne can be used for grazing, hay, green manure and cover crop. The primary use of lucerne is as feed for high-producing dairy cows, due to the high protein content.

Taraken (2014) set a series of experiments to measure the N accumulation by lucerne in the pasture. 12 treatments were set with four replicates a split plot randomised complete block design. The main treatment was P, S fertiliser and lime application in six different rates: control (0 lime/fertiliser), pelleted agricultural lime (L), S, and P (20% P-single superphosphate) + S (PS), L + S (LS) and L + S + P (LPS). Half of the S was applied as elemental S and the other half as gypsum. The application rates of fertilizer were 6000 kg L/ha, 128 kg S/ha and 50 kg P/ha. At the same time, a subplot treatment was applied as N fertiliser application rate: one-half of each main plot received a 0 or + N (urea 46% N) treatment after each harvest in the cut-and-carry system.

The result showed that general trend in soil mineralization N was a decline in both 0 N and + N plots during the experiment. The higher availability of soil N during late spring might cause by better soil conditions such as favourable soil moisture or temperature for mineralization. Although more soil mineralizable N occurred in winter, the soil condition is not available for plants to utilise N as they need, such as the low temperature resulted in plants became dormant. Cameron *et al*. (2005) also indicated that lower soil temperature during winter and early spring might cause slower N mineralization rate, plant growth, and pasture growth. The higher temperature and higher moisture of soil during the mid-summer to autumn resulted in the better conditions for soil N mineralization, which accelerated plant absorb N from soil, plant growth and pasture development (Moot *et al*., 2003).

In others work, Cameron (1992) reported that lucerne could fix N 125-600 kg N/ha/year, while Phillips (1980) indicated lower lucerne N fixation rates as 270 (rotating culture) and 300 (continuous culture) kg N/ha/year. In the Taraken (2014) experiment, the annual cumulative mean N fixed by

lucerne at the high end of the range of which Cameron (1992) reported. Cuttle *et al*. (2003) reviewed that the amount of annual N fixation by lucerne was about 22-25 kg N/ha/year for every tone of DM produced. The values of annual amount of N fixed by lucerne in the Taraken (2014) experiment from the 0 N plots was about 20-23 kg N/t DM/year, which was close to the range that Cuttle *et al*. (2003) reviewed. However, the N fixed from the +N plots in this experiment were much lower than 0 N plots, at 15-16 kg N/t DM/year.

2.5.3 Peas

Peas are the most commonly small spherical seed or the seed-pod of the pod fruit *Pisum sativum*. The colour of peas varies from green, golden yellow, or purple. Different estimated quantities of N fixed by peas have been reported. Virtanen and Saubert-Von Hausen (1952) estimated that peas could fix 65 kg N/ha. Lyon and Bizzell (1933) estimated 42-50 kg N/ha fixed by peas, grown as a companion crop with cereals. Mahler *et al*. (1979) reported the values of annual N fixed by peas is about 17 to 69 kg N/ha. Askin (1983) assessed the seasonal profiles of N fixation in eight cultivars of pea and found the greatest N fixation potential. The eight cultivars were replicated four times in a randomised block design. However, values of soil N accumulation by the eight cultivars were not measured.

Kumar and Goh (2000) reported the values of N fixation and accumulation of soil N and N balance for field pea. They also measured white clover as a comparison. They set the experiment at Henley Research Farm of Lincoln University in the Canterbury, New Zealand. White clover and field pea were sown in 12 main plots (35 x 20 m^2) per plots and applied fertiliser with 200 kg/ha single superphosphate before sowing. ^{15}N-labelled fertiliser was applied in a randomly placed micro-plot (1 x 1 m^2) in each main plot. Each of micro-plots received 30 at. % excess ^{15}N-enriched ammonium sulphate applied at 3.65 kg N/ha as a tracer. They also measure the N in weed to calculate the N fixation by legumes in the pasture. Table 2.4 shows the dry matter yield and N accumulation by white clover and field pea at final harvest.

Table 2.4 indicates similar amounts of plant dry matter accumulated by white clover and field pea, 15.0 and 15.3 t/ha respectively. White clover produced 0.3 t/ha seed (2% of crop dry matter yield), while pea produced 3 t/ha grain (20% of dry matter yield). Table 2.5 shows the amounts of total N accumulated, soil derived-N accumulated, biologically fixed N_2 in crops and crop components and N balance in white clover and field pea. It can be seen in Table 2.5 that field pea accumulated more N but fixed less N_2 than white clover. 365 kg N/ha accumulated by white clover as 74% tops N, 22% root N and 4% removed with seed. While, pea accumulated total N about 413 kg N/ha which partitioned into 61% tops N, 12% root N and 28% grain N. Compared with the values in Table 2.4, the proportion of N partitioned into seed which was described as N harvest index (NHI) was only 4 and

28% for white clover and field pea, respectively. However, both white clover and field pea were positive on N balanced. The net N gains contributed by N_2 fixation were both high as 313kg N/ha from white clover and 171 kg N/ha from field pea.

Table 2.4: Dry matter yield and N accumulation by white clover and field pea at final harvest. [a]Data followed by the same small or capital letters are not significant different at (P≤0.05) in a column within a crop or between crops, respectively, according to Tukey's LSD. [b]Data in parenthesis are proportions (%) of total DMY (harvest index) or N accumulation (NHI) (Kumar & Goh, 2000).

Crop and Components	DMY (t/ha)	Nitrogen accumulation (kg/ha)
White clover (*Wc*)		
Roots	4.7 a (31)b	81.1 a (22)
Shoots	10.0 a (67)	270.0 a (74)
Grain	0.3 (2)	14.1 (4)
Gcrop total (Wc)	15.0 aC (90)	365.2 aB (94)
Weeds (Wd)	1.7 b (10)	21.7 b (5.6)
Total (Wc+Wd)	16.7 a	387 a
Pea (*P*)		
Roots	2.9 b (19)	48.8 c (12)
Shoots	9.4 a (61)	249.8 a (61)
Grain	3.0 b (20)	114.4 a (28)
Gcrop total (P)	15.3 aC (96)	412.9 aA (97)
Weeds (Wd)	0.6 a (3.6)	14.4 a (3)
Total (P+Wd)	15.8 a	427 a

The dry matter yield of seed and tops for white clover agreed with those reported by other workers (Crush, 1987; Freeman, 1985). However, grain yield of field pea was lower than those reported by others (Kelstrup *et al*., 1996) at the same location by Kumar and Goh (2000). The damage of grain yield might cause by the birds. The total amount of N accumulated by white clover was like those reported by other workers in New Zealand, which clover accumulated N greater than 500 kg/ha (Crush, 1987). The values of NHI for peas as 28% was far less than other reported which was more than 60% in earlier studies (Amstrong *et al*., 1996; Haynes *et al*., 1993; Kelstrup *et al*., 1996). Field pea fixed much higher amounts of N about 286 kg N/ha than those reported by others. Beck *et al*. (1991) reported that 33-126 kg N/ha fixed by field pea. Evans *et al*. (1989) reported that the value of the amount of N was about 177 kg N/ha fixed by field pea. The higher values might have been caused by the inclusion of the root N in the Kumar and Goh (2000) study. That study indicated that the N present of the nodulated root had been ignored in most N_2 fixation studies and the contribution of the considerable amount of N which was released from the roots as rhizodeposition also has been ignored.

Table 2.5: Amount of total N accumulated, soil derived-N accumulated, biologically fixed N_2 in crops and crop components and N balance in white clover and field pea. [a] Data in parenthesis represent proportions (%) of total N accumulated (Kumar & Goh, 2000).

	Accumulated N (kg N/ha)	
	White clover	Pea
Total N accumulated	365	413
Tops N+root N	351 (96)[a]	299 (73)
Grain N	14 (4)	114 (28)
Soil N accumulated	38 (10)	127 (31)
Soil N retained by tops+roots	37 (10)	90 (22)
Soil N removed by grain	1 (<1)	37 (9)
Fixed N_2 accumulated	327 (90)	286 (69)
Fixed N_2 in tops+roots	314 (86)	208 (50)
Fixed N_2 removed by grain	13 (4)	77 (19)
N_2 fixed-total N removed (balance)	313 (86)	171 (42)

Table 2.5 shows the high correlation between DM yield or N accumulation and N_2 fixation significantly. Both crop DM and N accumulation can indicate the amount of N_2 fixed by these crops. Many other workers reported the marked correlations between DM yield and N_2 fixation for white clover (Goh *et al.*, 1995), subterranean clover (*T. subterraneum*) (Bolger *et al.*, 1995), lucerne (McCallum *et al.*, 1999) and grain legumes based pasture (Evans *et al.*, 1989). They also indicated that the correlations might not be so strong at where the pasture soil hold high plant-available mineral N or some other factors inhibited N_2 fixation. The comparison between white clover and field pea indicated the greater long-term benefit to N fertility of cropping soils provided by pasture legumes than grain legumes. Heenan *et al.* (1995) and Schultz (1996) also showed the similar results. And both legumes were more beneficial to cropping system than the non-legume system which only removed N from the pasture (McCallum *et al.*, 1999).

2.5.4 Other legume species

There are many other forage legumes researched in regards to N fixation in pasture land. Carlsson and Huss-Danell (2003) summarised the N fixation level of red clover (*T. pratense*) and lucerne. They reported that the magnitude of N_2 fixation could be up to 373 kg N/ha/year in red clover, and 350 kg N/ha/year in lucerne.

Table 2.6: Partition of accumulated N and amounts of soil N and fixed N_2 returned to the field as residues and removed in harvested product for the four legumes sown in spring 1988 and spring 1989 (Haynes & Williams, 1993).

Accumulated N	Spring 1988				Spring 1989			
(kg N/ha)	Lupin	Lentil	Field pea	Field bean	Lupin	Lentil	Field pea	Field bean
Total N accumulated	292	139	124	173	347	133	160	292
Root N	17	6	6	14	23	10	8	30
Stover N	55	27	26	61	46	33	36	49
Grain N	220	106	92	98	278	90	116	213
Soil N accumulated	181	107	96	125	221	101	137	194
Soil N returned	44	25	25	51	40	34	38	53
Soil N removed	137	82	71	74	181	67	99	141
Fixed N_2 accumulated	111	32	28	48	126	32	23	98
Fixed N_2 returned	28	8	7	24	39	9	6	26
Fixed N_2 removed	83	24	21	24	97	23	7	72
N_2 fixed-total N removed	-109	-74	-64	-50	-152	-58	-93	-141

Haynes and Williams (1993) set a series of experiment to study N accumulation form some field-grown legume crops. Table 2.6 shows the partition of accumulated N and amounts of soil N and fixed N. While field bean accumulated an intermediate amount and lentil accumulated the least amount. For all legumes in spring, the overall N balance was negative, which means N removed in the form of grain harvest greatly exceeded the quantity fixed by legumes. Although blue lupin produced highest yields and fixed the most N_2, blue lupin also had the most negative N balance in both years. At the same time, less N was removed from the lentil, field pea and field bean. The results also indicated that the reason for different N fixation rate in different legume might be the different content of plant lignin and polyphenol in legume species.

To compare with others' studies, Table 2.7 summarises values of N_2 fixation (kg N/ha/year) in other legume species. Data are listed according to decreasing latitude. Swards were kept undercutting management, except when noted 'Grazing'. Nitrogen fixation rate for those perennial forage legumes affected by many factors, such as plant age, plant density, crop latitude and measure methods. Compared with those legumes in Table 2.7, white clover, red clover and lucerne can fix more N than others. Silver and Hardy (1976) also reported greater quantities of N fixed by forage legumes on average than grain legumes. The good correlation between N fixation amount and soil management in different studies suggests that managements favouring legume productivity should ensure high N_2 fixation (Carlsson & Huss-Danell, 2003).

Table 2.7: Summary of N2 fixation (kg N/ha/yr) in other legme species. Data are listed according to decreasing latitude. Swards were kept under a cutting management, except when noted 'Grazing'. [a]ID - ^{15} N isotope dilution; NA – ^{15}N nature abandance; ND- nitrogen difference. [c]Indicates reference crop. [d]Above and below-ground harvests included. [e]Noninoculated, nonodulating or ineffective plant variety. [f]Indicates that reference crop is in monoculture (Calsson and Huss-Danell, 2003).

Species	Range	Method[a]	Cause of variation	Stand description and reference crops	Geographic location,	Reference
Melilotus officinalis	4–123[d]	ID, ND	2 years, reference crop, method, site	Monoculture, *Medicago officinalisce* , *M. sativace* , *Brassica oleraceac* , *Hordeum vulgarec*	Alaska, 64° N	Sparrow et al., 1995
Vicia faba	82–249[d]	ID, ND	3 years, reference crop, method, site	Monoculture, *Vicia faba*[ce], *M. sativa*[ce], *Brassica oleracea*[c] , *Hordeum vulgare*[c]	Alaska, 64° N	Sparrow et al., 1996
Pisum sativum	42–144[d]	ID, ND	4 years, reference crop, method, site	Monoculture, *Pisum. Sativum*[ce], *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[e]	Alaska, 64° N	Sparrow et al., 1997
Lens culinaris	21–91[d]	ID, ND	5 years, reference crop, method, site	Monoculture, *Lens culinaris*[ce] , *M. sativa*[ce], *Brassica oleracea*[c] , *Hordeum vulgare*[c]	Alaska, 64° N	Sparrow et al., 1998
Lupinus alba	33–202[d]	ID, ND	6 years, reference crop, method, site	Monoculture, *Lens culinaris* ce , *M. sativa*[ce], *Brassica oleracea*[c], *Hordeum vulgare*[c]	Alaska, 64° N	Sparrow et al., 1999
Vicia faba	160–238	ID, ND	Reference crop,method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51° N	Witty, 1983a
Pisum sativum	53–97	ID, ND	Reference crop,method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51° N	Witty, 1983a
Phaseplus vulgaris	84-131	ID, ND	Reference crop,method	Monoculture, *Lolium perenne*[c], *Brassica rapa*[c]	England, 51° N	Witty, 1983a
L. corniculatis	25–130[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45° N	Heichel and Henjum, 1991
L. corniculatis	49–112[d]	ID	4 years	Mixture with *Phalaris arundinacea*[c]	Minnesota, 45° N	Heichel et al., 1985
T. subterraneum	50–150	NA	3 years	Mixture with *Phalaris arundinacea*[c]. Grazing	Australia, 35° S	Dear et al., 1999
T. subterraneum	30–170	NA	3 years, plant density	Mixture with *M. sativa*, *P. arundinacea*[cf] . Grazing	Australia, 35° S	Dear et al., 1999
T. subterraneum	20–100	NA	3 years, plant density	Mixture with *Phalaris arundinacea*[c]. Grazing	Australia, 35° S	Dear et al., 1999
Pisum sativum	286[d]	ID		Monoculture, weeds[c]	New Zealand, 43° S	Kumar and Goh, 2000

Allos and Bartholomew (1959) showed the N fixation performance affected by different levels of N fertiliser applications in 1958. They studied about five different legumes: soybean (*Glycine max*), lucerne, sweet clover (*Melilotus spp*), white clover and birdsfoot trefoil (*Lotus corniculatus*). Table 2.8 shows the N fixation about different levels of available N and total plant growth in legumes grown for ten weeks. The significant increase occurred in the growth of legumes by the rise of fertiliser application rate and the excess N application replaced fixation process by legumes which means that fertiliser N application stimulated growth and need for N. In addition, the highest rate of N application may not produce maximum possible growth and N uptake in all the legume studies which the exception occurred in birdsfoot trefoil. They also reported that total fixation of N by some legumes has a great correlation to the amount of N. Soybeans fixed the largest amount of N, followed by lucerne, sweet clover, white clover and birdsfoot trefoil.

Table 2.8: Nitrogen fixation in relation to level of available N and total plant growth in legumes grown for a period of 10 week. * Corrected for the nitrogrn supplied by the gravel and seed (Allos & Bartholomew, 1959).

Nitrogen Additions Per Pot	Weight Per Pot			N Uptake Per Pot			N From Fertiliser	N Fixed From Air *	Percent of Total Fixation
	Top	Root	Total	Tops	Roots	Total			
mg.	g.	g.	g.	mg.	mg.	mg.	mg.	mg.	%
Soybean									
0	43.1	9.0	52.1	1336	320	1656	0	1639	100
80	46.9	9.3	56.2	1484	293	1777	68	1692	95
320	65.3	11.2	76.5	2118	394	2512	252	2243	89
560	70.3	11.9	82.2	2290	376	2666	464	2185	82
800	93.5	13.8	107.3	2666	422	3088	648	2423	79
Lucerne									
0	9.1	6.1	15.2	320	193	513	0	496	100
80	12.0	7.2	19.2	413	202	615	73	525	85
320	12.9	7.0	19.9	428	203	631	285	329	52
560	15.5	8.9	24.4	505	266	771	472	282	37
800	20.3	12.1	32.4	643	308	951	725	209	22
Sweet clover									
0	8.6	3.3	11.9	312	135	447	0	430	100
80	11.4	3.6	15.0	45	149	194	73	494	85
320	13.8	6.2	20.0	522	209	731	261	453	62
560	20.7	14.0	34.7	639	330	969	505	464	48
800	23.4	16.1	39.5	718	409	1127	756	354	31
White clover									
0	5.0	1.2	6.2	165	40	205	0	188	100
80	7.6	1.6	9.2	262	52	314	63	234	75
320	12.0	2.4	14.4	368	90	458	282	159	35
560	13.5	3.0	16.5	547	95	642	527	98	15
800	16.7	3.5	20.2	593	115	708	609	82	12
birdsfoot trefoil									
0	4.6	1.5	6.1	142	52	194	0	177	100
80	6.3	1.6	7.9	186	59	245	73	155	63
320	8.5	1.9	10.4	265	59	324	246	61	19
560	9.2	1.2	10.4	309	40	349	329	3	1
800	9.5	1.4	10.9	346	50	396	375	4	1

2.6 Perennial Lupin

2.6.1 Introduction

Lupinus is a genus of flowering plants in the legume family, Fabaceae. There are over 200 species in the genus. The species are mostly herbaceous perennial plants about 0.3-1.5m tall, while some of them are annual plants and a few are shrubs up to 3m tall (Villa-Ruano *et al*., 2012). Seeds of some lupin species have been used as food for ruminants for many thousands of years. Due to its perennial character, perennial lupin has a competitive advantage over shorter-lived associated species. Perennial lupin is available to grow in acidic soil (pH < 5.5) which may have high levels of

exchangeable Al and the level is toxic to clovers and lucerne (soil Al >3 ug/ml), and low soil P level (Olsen P <10 ug/ml). The growth ability of lupin indicates that it is better adapted to acid soil environment without large inputs of lime and fertiliser than other conventional pasture and forage legumes. It has been reported that perennial lupin seeds are assessed better than soybeans for health benefits, due to the similar protein content and less fat than soybean and lupin seeds are also gluten-free, high in dietary fibre, amino acids, and antioxidants (Ross, 2011). The utilisation of lupin seeds for human food is currently small, but some species of lupin are highly regarded as fodder, especially for ruminants (Ross, 2011). Like other legumes, perennial lupin can fix N from atmosphere into NH_3 to increase the soil fertility, nodulated by *Bradyrhizobium* soil bacteria (Kurlovich, 2002). Black *et al*. (2015) reported that bradyrhizobia with a distinct nodA gene nodulate *Lupinus polyphyllus* in New Zealand soils.

2.6.2 Lupin in New Zealand agriculture

Scott *et al*. (1995) reported the studies about the forage species suitability of perennial lupin as a forage species in the New Zealand high country. Perennial lupin is considered as the most appropriate and productive species to grow by the assessment of the soil conditions and climate for legume pasture. Ryan-Salter *et al*. (2013) reported that there are currently three perennial lupin species in New Zealand: *L. polyphyllus, L. arboreus, L. perennis*. The latter two species were not being evaluated as forage species in New Zealand. The species that is referred to as perennial lupin in this thesis is *L. polyphyllus*. The other two annual species of lupin, *L. angustifolius* (blue lupin) and *L. luteus* (yellow lupin) have been used for forage species in New Zealand but mostly subsequently replaced with increased use of lucerne between the 1940's and 1950's (Greenall, 1956; McPherson, 1940). Therefore, *L. polyphyllus* is a species of perennial lupin which has potential as a forage species to adapt to low pH, low P, high soil Al and wet areas. *L. polyphyllus* is also the species which has been most studied in New Zealand recently (Ryan-Salter *et al*., 2013).

L. polyphyllus is often referred to as Russell lupin, which grows up to 1.5 m tall and dies back to a stout crown each winter. Some multi-coloured form is a hybrid selected by George Russell in the UK and was introduced into New Zealand as a garden horticultural species. *L. polyphyllus* has greater tolerance of acidity and exchangeable Al than other pasture legumes (Scott *et al*., 1995; White, 1995) and produce more biomass on low fertility soil than other pasture legumes (Davis, 1981b). Davis (1981b) showed that the ability of perennial lupin to grow on acid soil with pH 5.0, where the soil has high levels of soluble Al and low soil P limitation that clover cannot thrive.

In the study of Davis (1991), eight other legume species were investigated under nine different P application rates ranging from 0 to 800 kg P/ha. The growth of lupin showed no positive response to S and P. However, Svavardottir *et al*. (1994) reported the growth of browntop (*Agrostis capillaris*)

and sweet vernal (*Anthoxanthum odoratum*) along the whole P gradient 13 years later, which indicated the good performance of lupin fixing N by P application. At the same time, white, red and alsike clovers only thrived at high P rate applications, which indicated the capable of extracting soil P by lupin but not available to the clovers (White, 1995). White (1995) also indicated the higher acid tolerance of perennial lupin than many other traditional pasture forage legumes.

Perennial lupin has alkaloids in its herbage, which increases in concentration in summer with the growth of flower and seed pods. The increase of alkaloid allows some seed to mature and the alkaloid content of perennial lupin is sufficient to inhibit rapid herbage intakes by ruminants due to the bad taste for ruminants which also give a resilience against any insect pests. However, the mature seed is unlikely to be eaten which is protected by high alkaloid content (Gibbs, 1988).

In the South Island of New Zealand, the conventional forage legumes, white clover and lucerne covering this area were limited by soil and climate conditions (Scott *et al*., 1995). Thus, compared with those legumes, perennial lupin is better suited to dry, infertile soil. Black *et al*. (2014) compared the productivity between perennial lupin-cocksfoot (*Dactylis glomerata*) pasture with lucerne pasture at Lincoln University, Lincoln, New Zealand. The results indicated that the lupin-cocksfoot pasture was about 65-70% as productive as the lucerne pasture in the first year which supported using perennial lupin-cocksfoot pasture as an alternative forage option in the unsuitable grassland for lucerne.

Table 2.9: Change in species dominance over six periods in 25 years related to fertiliser levels (superphosphate in kg/ha/yr: 1 = nil, 2 = 50, 3 = 100, 4 = 250, and 5 = 500 + irrigation) and grazing management (H = high stocking rate, M = moderate, L = low, and s = set stocking and m = mob stocking). A = alsike clover, (*Trifolium hybridum*), C = chewings fescue (*Festuca rubra*), D = cocksfoot (*Dactylis glomerata*), H = Hieracium (*Hieracium pilosella*), K = Caucasian clover (*T. ambiguum*), L = lupin (*Lupinus polyphyllus*), O = tall oat grass (*Arrhenatherum elatius*), W = white clover (*T. repens*), and Z = fescue tussock (*Festuca novae- zealandiae*) (Scott, 2008).

Grazing	Year 2-4					Year 5-8					Year 9-12					Year 13-16					Year 17-20					Year 21-24				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Hs	L	A	A	L	A	H	A	A	H	W	H	L	K	K	C	H	K	K	K	C	H	K	K	K	C	H	K	K	K	C
Hm	L	L	L	L	W	L	L	L	L	L	H	L	L	L	K	L	K	K	L	K	L	K	K	K	K	L	K	K	K	K
Ms	L	L	A	L	A	H	L	L	AW		H	L	L	K	C	H	L	L	K	C	H	L	L	K	C	H	L	L	K	C
Mm	L	L	L	L	A	H	L	L	L	D	H	L	L	L	D	H	L	L	L	C	H	K	K	K	K	H	O	O	K	C
Ls	L	L	L	L	A	H	L	L	L	D	H	L	L	L	C	H	L	L	L	C	Z	K	K	K	K	Z	O	O	K	C
Lm	L	L	A	L	A	H	L	L	L	L	H	L	L	L	L	Z	L	L	L	C	Z	O	O	O	K	Z	O	O	O	C

Scott (2008) showed the change in species dominance over six periods in 25 years related to fertiliser levels and grazing management. Table 2.9 shows the significant competitive advantage of perennial lupin in a long-term experiment. Particularly, lupin became dominant in the pasture and remained

dominant at the lower fertiliser levels which indicated the high tolerance of the low fertiliser and soil acidity.

Nordmeyer and Davis (1977) reported the changes of dominant species in grassland was the process of a cycle of organic matter and N accumulation to new equilibrium level (Figure 2.5). To reach the new equilibrium, a continuing source of N, P and S will be necessary. When one legume species cannot be adapted to all processes of changing fertility and competition, the efficient N fixation by other legume species is necessary. The perennial legume is a better choice to provide the great competition to attend the new equilibrium level.

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Figure 2.5: Accumulation of N after 5, 14, 21 and 30 years in different ecosystem compartments in a revegetation succession of sown and volunteer legumes on eroded subsoil at Hut Creek in the Craigiebum Forest (Note That the time scale is not strictly drawn) (O'Connor & Nordmeyer, 1996).

O'Connor and Nordmeyer (1996) also reported the great competition of perennial lupin in the N accumulation during the revegetation succession. Figure 2.6 showed the increasing amounts of N accumulation in different compartments of the ecosystem as a succession of legumes occurred on eroded subsoil at Hut Creek in the Craigieburn Forest, South Island. The leading position of sown clover for the first 3 to 4 years was replaced by a succession of volunteer lotus for another 9 or 10 years. Then, the lupin showed the great competition for the next 16 years, which has progressively adapted to the initial decline in applied P fertility and residual exchangeable Al. The increase of total N accumulation was mainly lead by legumes. The changes were as the same trend as Nordmeyer and Davis (1977) reported. The authors also indicated the specific beneficial effect than clovers under the low-level P application and lupin can enhance the topsoil level of exchangeable Ca also reducing the level of exchangeable Al. Bryant (1974) reported that in acidic soils the relative dominance of the species might be related to its ability to compete for nutrients which may also be a reason for the changes of dominant species.

Perennial lupin also has other advantages for pasture production for cattle and sheep. Black *et al*. (2014) reported the sheep performance on perennial lupin over 3 years at Sawdon Station, which is a 7100 ha farm near Lake Tekapo in the Makenzie basin. The experiment compared the performance of Merino ewes grazing on a perennial lupin pasture with lucerne and clover-based pastures (control). The results showed that the lambing percentage was about 111% for Merinos on Perennial lupin which was little higher than for the Merinos on the lucerne and clover-based pastures. The ewes averaged 58 kg for the lupin pasture and 62 kg for lucerne pasture.

Figure 2.6 shows N concentrations of perennial lupin on Sawdon Station by Black *et al*. (2015). In Figure 2.6, the N content was significantly highest in the leaves and flowers plus green seed pods of lupin and lowest in the petioles, stems and dead fractions. The general decrease occurred in these parts of lupin except in flower plus green seed pods. The N content showed high values in the flowers plus green pods, while declines were demonstrated in the petioles and stem as the plants developed. The results explained the reason for more leaves and flowers were grazed by sheep which expected by Kitessa *et al*. (1993) that the sheep adapt to lupin.

Image removed for Copyright compliance

Figure 2.6: Nitrogen concentrations of perennial lupin on Sawdon Station. Error bars are standard errors of means (Black *et al*., 2014).

The results from the Sawdon Station experiment showed that perennial lupin was used successfully as a forage crop for lambing which also provided tall shelter for new-born lambs. The perennial lupin pasture also provided acceptable lambing rates, lamb weights and wool production compared with control pasture. The high N content crude protein (N% × 6.25) was shown in lupin during the grazing season. Overall, the experiment also indicated the possibility of perennial lupin as an alternative to lucerne for high country pastures in New Zealand.

Scott and Covacevich (1987) reported that perennial lupin could not survive by continuous set stocking that lax grazing at low to moderate stocking rates is probably preferred. Svavarsdottir (1995) reported the height of lupin growth at flowering about a meter which results in a pronounced shading effect on prostrate, light-demanding plants, such as mouse-eared hawkweed (*Hieracium pilosella*). The growth of mouse-eared hawkweed can be significantly reduced by the low sunlight and the leaves of surviving plants grow more vertically, seeking light, and are thus able to be grazed by livestock. At the same time, the tall height of perennial lupin brings less likely to be shaded by the associated grasses than prostrate legumes such as white clover (White, 1995).

Scott (2003) indicated that because of the wide seasonal and annual variation of pasture forage legumes in dryland environments, the long-term perspective on sustainability issues for legumes should be considered to protect the high pasture productivity. The dryland environment has the great challenge about moisture, low fertility, low soil pH and high Al toxicity for conventional pasture legume thrive (Brock *et al*., 2003; Brown *et al*., 2009). However, perennial lupin has the advantage to dryland environment which shows it has the potential as an alternative legume to conventional pasture legumes.

2.6.3 Lupin and soil N accumulation

Like other legumes, perennial lupin can fix N2 from the atmosphere into their tissues. Annual N fixation by lupin species is estimated to about 145 to 208 kg N/ha (Nutman, 1976). And Rizk (1966) reported higher quantity of N fixed by blue lupin (*Lupinus angustifolius*) than peas with an average of 176 kg N/ha. Haynes and Williams (1993) also reported the 292 and 347 kg N/ha total annual N accumulated by blue lupin showed in Table 2.6. And Sparrow *et al*. (1995) showed 33-202 kg N/ha/year values of N fixation by lupin (*Lupinus alba*) in Table 2.7. The high N yield of lupin can be exploited by planting lupin in rotation with grasses or cereal crops. Rhodes (1980) showed that the amount of N Fixed by blue lupin (cv. Uniharvest). The nitrogen fixation by blue lupin was about 183 kg N/ha which was more than the double amount fixed by pea (*Pisum sativum*). In addition, the N concentration in annual ryegrass (*Lolium multiforum* cv. Tama) was higher after blue lupin than after pea, which show the higher N fixation ability by blue lupin than peas. Rhodes (1980) also reported the N yield of blue lupin biomass at 43 days after sowing. Although N yield at final harvest was lower than the maximum recorded, the maximum N yield of blue lupin was 240 kg N/ha which was considerably higher than peas cv. Huka and cv. Puke. For blue lupin, seed N yield was the largest component of biomass N, which the value of N harvest index was 0.91. And the proportion of biomass N yield was about 4%. As get, the contribution of perennial lupin to soil N accumulation has not been investigated.

2.7 Soil Carbon in pasture system

Carbon in the soil has two forms, inorganic as carbonate minerals and soil organic matter. Soil C stock plays a key role in C cycles which the small changes can cause large changes in global climate, such as transfer land use from forest to city reducing the ability of CO_2 absorption resulting in higher atmosphere temperature.

2.7.1 Soil organic matter

Soil organic matter includes soil microbes, dead and decaying plant and animal remains, and the highly decomposed material from fresh plants known as humus, affected by climate, soil acidity, soil conditions and human activity (McLaren & Cameron, 1996). Soil organic matter is a critical component of soil affecting soil physical, chemical and biological properties. Soil organic matter contributes to remain nutrients and water, reduce erosion and improve soil structure resulting in better soil quality and greater soil productivity (Ontl & Schulte, 2012).

2.7.2 Soil C stock

Soil C sequestration the process removing CO_2 from atmosphere and storing in soil C pool, mainly by plant photosynthesis (Ontl & Schulte, 2012). Many factors affect soil C stock in pasture system,

natural and/or anthropogenic, such as, climate, fertiliser application (McSherry & Ritchie, 2013), grazing intensity (Barnett *et al*., 2014), irrigation rate *(Condron et al., 2014; Kelliher et al*., 2012) and plant cultivation (Rutledge *et al*., 2014). The pasture managements aim to increase pasture production, which shows land-use intensification in New Zealand and also worldwide (MacLeod & Moller, 2006). In pasture system, C is fixed by photosynthesis and combines into the soil followed by plant senescence or dung deposition. Then, carbon loses from soil stock by respiration, leaching and pasture animal produce (Schipper *et al*., 2007). The net balance of the C depends on these input and outputs shows the changes of soil C stock (Kirschbaum *et al*., 2015).

In New Zealand, Schipper *et al*. (2007) indicated a series studies about soil C stock losses in continued grazing pasture on dairy farms, sheep and beef farms (Schipper *et al*., 2010) in flat land. However, soil C stock showed an increase in high country soils.

Soil changing can be more complex in hill country soil than flat land soil caused by a range of greater erosional processes on slopes. There are two possible causes for the increase in soil C for high country soil. Firstly, hill country forest was cleared and transferred to hill country pasture, resulted in topsoil loss causing the decrease in soil organic matter. Secondly, P fertiliser application increasing the legume growth, N fixation and pasture production in the 1950s and 1960s as aerial topdressing (Haynes & Williams, 1993). Parfitt *et al*. (2013) also concluded the increase of soil C mainly caused by C input and N fixation with a less contribution of redeposited topsoil by modelling the C accumulation in hill country pasture. Schipper *et al*. (2014) also demonstrated that net N immobilisation is like to decline in next few decades resulted from N saturation of soil organic matter both in hill country and flat land with clear evidence.

Although many studies illustrated the soil C changes by various factors, we know a few about the scale of soil C changes and the association of the changes. The further study may establish more measurements in pasture system to understand soil C stock changes.

2.7.3 C: N ratio

In the soil C cycle, many other nutrient elements such as N, P and S are present in soil organic matter turnover process and participate in the cycles, separately but closely related. The C: N ratio is a relative proportion in organic material and is used as a guide in describing the stage of decomposition and mineralization of N from organic residues. The C: N ratio in soil (from 4: 1 to 9: 1) is much lower than in plant (from 20: 1 to 100: 1) (McLaren & Cameron, 1996).

2.8 Conclusions

Nitrogen is important for soil, plants and animals. The N cycle in the grazing pasture provides a transfer between organic N and inorganic N. Soil N accumulation affected by climate, age of the pasture, fertiliser application and other soil management. Soil N content increases naturally by the soil development without plant harvest remove in unfertilised soil. In addition, the content of N decreased by the increase of soil depth. Soil N accumulation not only increased by fertiliser N application rates, but also affected by other (P, S) fertiliser application rates. Different legumes have various abilities to N fixation. The values of annual N fixation by clover strongly influenced by the soil conditions and fertiliser applications, ranged from 17 to 700 kg N/ha. The influences are similar to N accumulation by lucerne and peas, ranged from 125 to 600 kg N/ha/year and from 17 to 171 kg N/ha/year, respectively. Although other legumes can also increase soil N content by fixing N from the atmosphere, the amounts of N accumulation are generally lower than those forage legumes and not be used for pasture grazing. Lupin species accumulates N from soil like other legumes, but hold the higher tolerance of acid soil, toxic Al and low soil P level than other legumes (such as clovers, lucerne). Except for the great adaptable ability; perennial lupin also shows a competitive advance due to its perennial character. In New Zealand, some workers studied for the ability of N fixation by some lupin species and N yield in different plant parts, but no study for the soil N accumulation trend by the perennial lupin (*Lupinus polyphyllus*) which this is also the objective of this study.

Chapter 3

Field Study

3.1 Introduction

This field soil experiment that involved sampling perennial lupin (*Lupinus polyphyllus*) stands of varying ages. The soils were collected from eight sites across four South Island high country farms: Sawdon Station, Glenmore Station, Omarama Station and Dasher Station and from one site at Lincoln University, Lincoln. A summary of site information is presented in Table 3.1. Plates 3.1 to Plate 3.5 show the growth of perennial lupin at the different sites. Soil samples for the field study were collected on 12 June 2017 and transported back to Lincoln University. Site codes used in this study were: site 1 (Glenmore Station), site 2 (Glenmore Station Original Experiment), site 3 (Glenmore Station New Experiment), site 4 (Mount John Station), site 5 (Sawdon Station), site 6 (Omarama Station), site 7 (Dasher Station) and site 8 (Lincoln University).

3.2 Materials and meathods

3.2.1 Soil collection

Table 3.1: The names, locations, soil conditions, fertiliser histories, plant history and climate information of eight sites collected across four farms (Sawdon Station, Glenmore Station, Omarama Station and Dasher Station) and the Lincoln University Campus.

Site code	Site	Location	Soil type	Fertilizer History	Plant History	Annual Rainfall (mm)	Altitude (masl)
1	Glenmore Station	Tekapo	Brown	Low-med	Lupin/cocksfoot	590	700
2	Glenmore Original Exp.	Tekapo	Brown	Low-med	Lupin/native grass	590	720
3	Glenmore New Exp.	Tekapo	Brown	Super	Lupin	590	650
4	Mount John Station	Tekapo	Brown	Low	Lupin	590	700
5	Sawdon Station	Tekapo	Brown	Nil/Recent	Lupin/cocksfoot/ native grass	600	700
6	Omarama Station	Omarama	Recent	Nil	Lupin	400	490
7	Dasher Station	East Otago	Brown	Nil	Lupin	1000	450
8	L.U. Campus	Lincoln University	Templeton silt loam	Low	Lupin/cocksfoot	640	9

3.2.2 Experimental design

The experimental designs differed across the eight sites. Some of them were existing plot experiments, some of them were large-scale paddock experiments and others were areas with randomly growing plants. Varying aged perennial lupin was located at these different sites. The histories and scales of sites sampled in this study were also different. The Glenmore Station sites were the large-scale paddock experiments, Omarama sites and Dasher Station sites were small plots , and Sawdon Station and Lincoln University Campus site had randomly growing plants. Neighbouring pasture of each site was also sampled (4 reps/site). Site 1, 2 and 3 lupins stand soils were compared with the same pasture soil, due to the three sites having similar locations. And other lupin stand soils of the other site were compared with their own pasture soils.

The same number of samples were collected in adjacent pasture sites, with ten replications of each sample. All soils were core-sampled to a depth of 15cm, using a 2.5 cm diameter soil corer. Then the soil cores were cut in half with a small knife at 7.5 cm to provided two soil depth: 0-7.5 cm is the depth A, while 7.5-15 cm is depth B. All sites were sampled with two depths except Omarama Station. The soil condition of Omarama site was stoney, so only the A depth was sampled for both the lupin stand soils and pasture soils.

There were two main sampling methods depending on the lupin site scale. The soil sampling method of “large areas” is shown in Figure 3.1. Each lupin paddock and pasture paddock was around 4-20 ha with an average of 10 transects. Each transect was 50m long and was using logged using Global Positioning System (GPS). Twenty soil cores were sampled along each transect and bulked. Individual soil cores were taken 10 cm away from the crown of the lupin plant to ensure that rhizosphere or “rhizosphere influenced” soil was being sampled. For the sites with random growing type perennial lupin, the soil core was also taken 10 cm away from each lupin crown with twenty replications.

The soil sampling method for plot experiments is shown in Figure 3.2. There were 30 plots of equal size, 5m x 3m. Plots with typical plant numbers were selected for sampling along a 2.5 m transect. Ten soil cores were sampled in each transect and also each soil core was taken 10 cm away from the lupin crown.

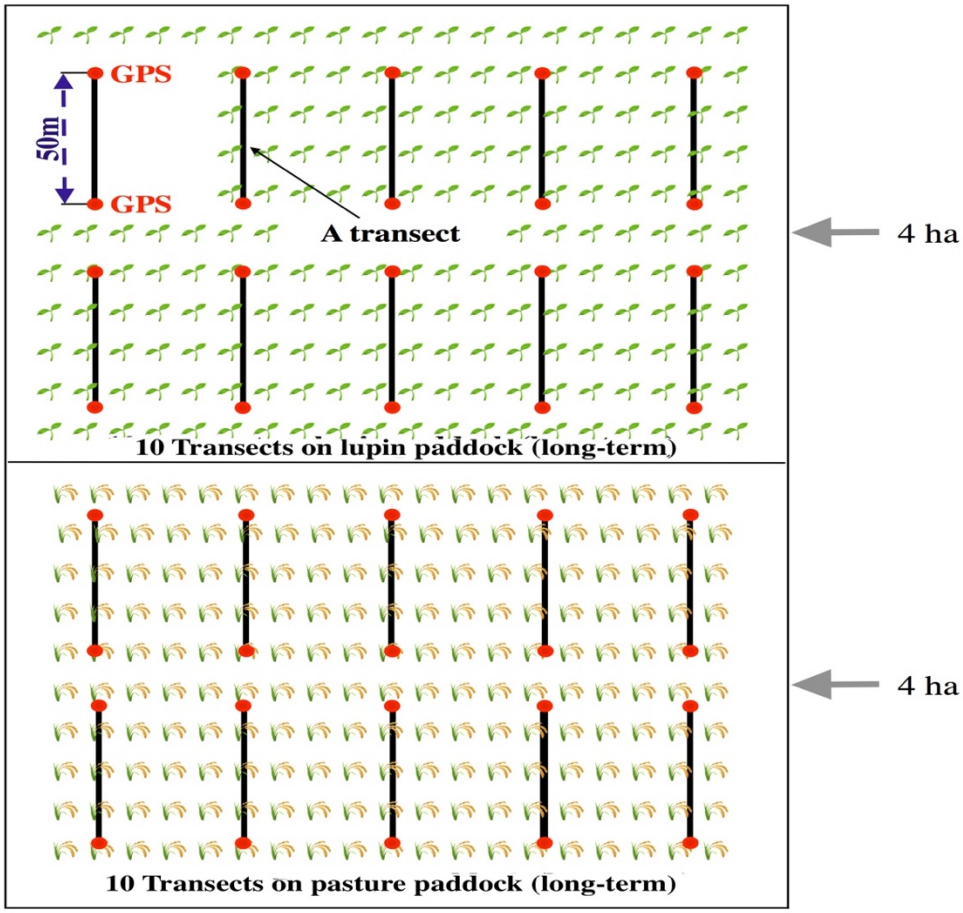


Figure 3.1 Soil sampling method used of large (paddock) areas at Glemmore Station (site 1 and 2).

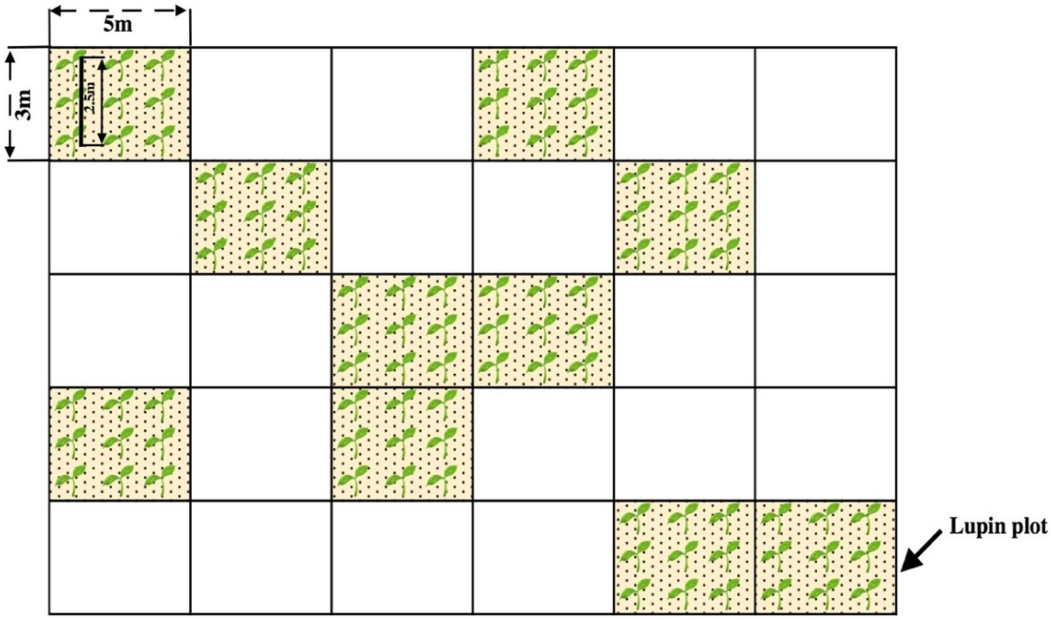


Figure 3.2 Soil sampling method used of small (established) plot experiments at Glenmore Station New experiment site (site 3), Omarama Station (site 6) and Dasher Station (site 7).

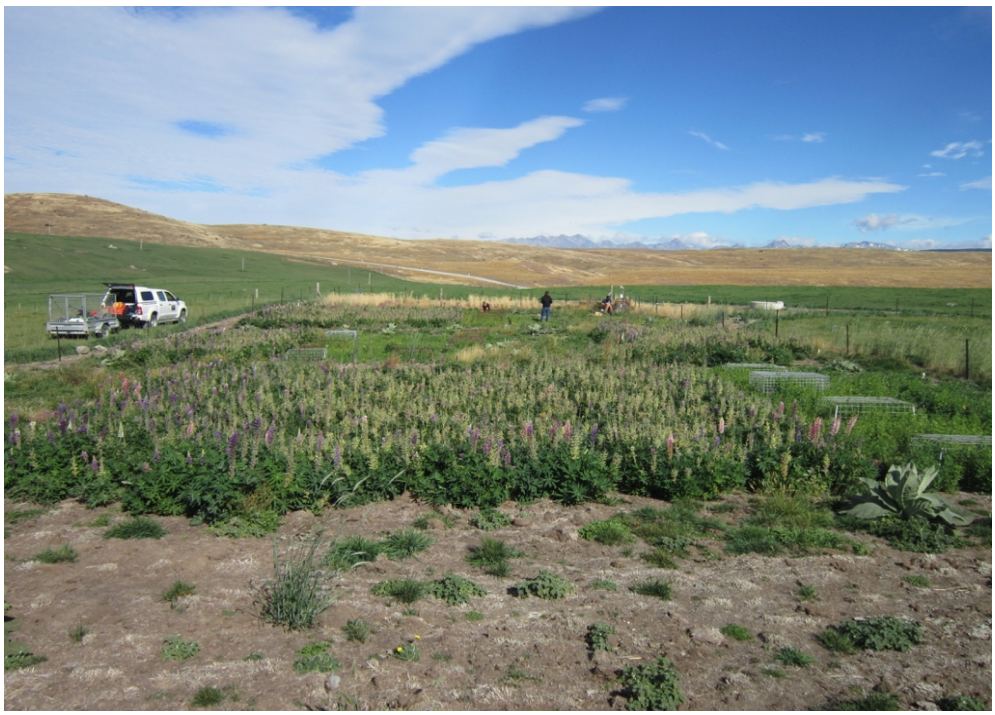


Plate 3.1: Perennial lupin experiment at Glenmore Station (site 3). Photo credit: Daniel Martin-Hendrie.

Plate 3.2: Perennial lupin stand with Merino sheep at Sawdon Station (Site 5). Photo credit: Alistair Black.

Plate 3.3: Perennial lupin experiment at Dasher Station (site 7). Photo credit: Daniel Martin-Hendrie.

Plate 3.4: Perennial lupin-cocksfoot experiment at Lincoln University (site 8). Photo credit: Alistair Black.

Plate 3.5: Perennial lupin experiment at Omarama Station (site 6). Photo credit: Daniel Martin-Hendrie.

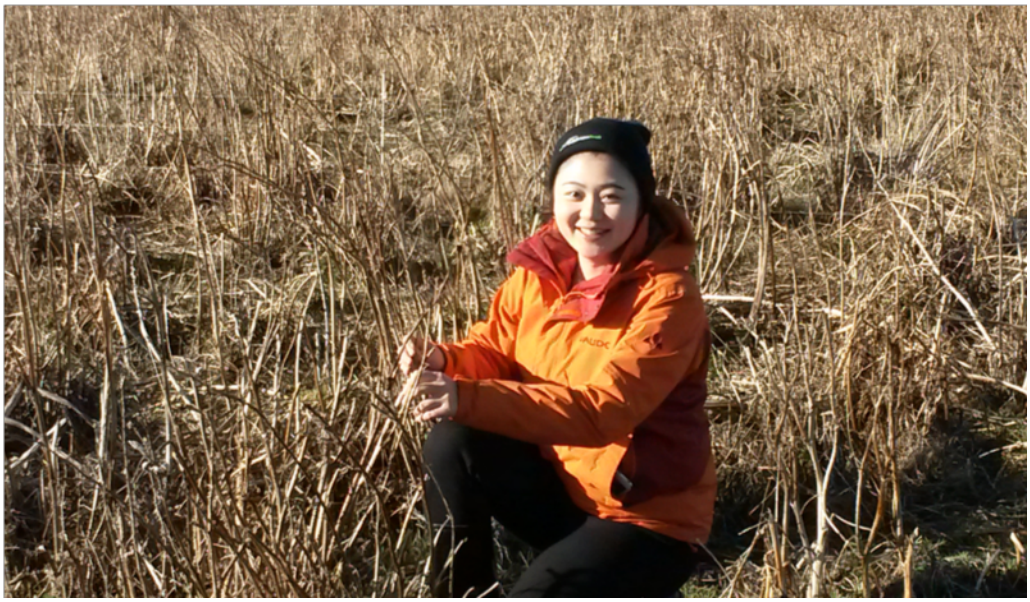


Plate 3.6: Lupins study for field study on 13 June 2017 in winter, Sawdon Station (site 5). Photo credit: Jim Moir.

3.2.3 Soil chemical analysis

All soil samples were analysed for total soil N, total soil C, mineralizable N. Two bulk samples were analysed for soil pH, Olsen P, sulphate S and total organic C (TOC). In field experiment and glasshouse experiment, soil pH was measured at a water: soil ratio of 2.5: 1 (Blakemore et al., 1972). The Olsen P was measured by using the method of Olsen *et al*. (1954). Extractable soil sulphate was measured by the method of (Searle, 1979). A method of Keeney and Bremner (1966) was used to measure soil mineralizable N. The methods to analyse the soil nutrient are presented in Table 3.2.

Table 3.2: The methods of analyses used in field study.

Analysis	Method
Total soil N and C	Horneck and Miller (1998)
Mineral N and mineralizable N	Keeney and Bremner (1966)
Soil pH	Blakemore *et al*. (1972)
Olsen P	Olsen *et al*. (1954)
Sulphate S	Searle (1979)

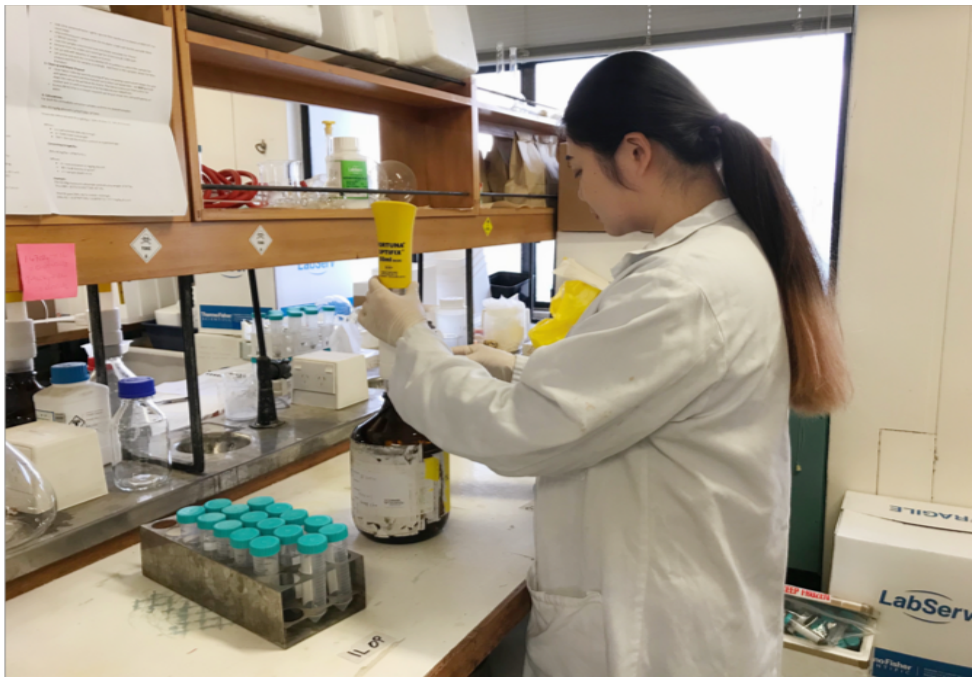


Plate 3.7: Soil mineral N and mineralizable N analysis in Lincoln University Burns Building. Photo Credit: Kevin Zhou.

3.2.4 Soil pH, Olsen P and sulphate S

Soil samples were taken from the eight sites transferred to Lincoln University. Soil samples were labelled and sent to the commercial laboratory ARL (Napier, Hawke's Bay, New Zealand) for analysis.

Table 3.3: Soil analysis results for soil pH, Olesn P, sulphate S for perennial lupin and pasture selected from eight sites. Soil depth 'A' = 0-7.5 cm horizon, soil depth 'B' = 7.5-15 cm horizon.

Site	Forage Type	Soil Depth	Core Length	Soil pH	Olsen P	Sulphate S
			cm		ug/ml	ug/g
Glenmore	Lupin	A	7.5	5.5	22	6
		B	7.5	5.2	10	7
Glenmore Old	Lupin	A	7.5	5.6	41	9
		B	7.5	5.2	21	7
Glenmore Wild	Lupin	A	7.5	5.3	40	2
		B	7.5	5.1	26	2
Glenmore Pasture	Pasture	A	7.5	5.4	31	26
		B	7.5	5.4	12	12
Glenmore Daniel	Lupin	A	7.5	5.0	49	29
		B	7.5	5.2	16	14
	Pasture	A	7.5	5.1	12	9
		B	7.5	5.3	6	4
Sawdon Station	Lupin	A	7.5	5.2	21	10
		B	7.5	5.8	15	7
	Pasture	A	7.5	5.9	8	1
		B	7.5	5.9	5	<1
Omarama	Lupin	A	7.5	5.6	29	14
	Pasture	A	7.5	6.0	16	3
Dasher Station	Lupin	A	7.5	4.8	18	31
		B	7.5	5.2	8	14
	Pasture	A	7.5	4.8	15	8
		B	7.5	6.0	11	6
Lincoln University	Lupin	A	7.5	6.1	18	2
		B	7.5	6.1	22	<1
	Pasture	A	7.5	5.8	15	5
		B	7.5	6.0	39	<1

3.2.5 Statistical analysis

All data sets were analysed to test for treatment effects by conducting an analysis of variance (ANOVA) using GENSTAT 16 (Lawes Agricultural Trust, Rothamsted, UK). For the field study, the

treatment effects analysed by ANOVA were total soil N, total soil C, soil mineral N, and soil mineralizable N.

3.3 Results

3.3.1 Total soil N and C

Total soil N showed large variation between sites. Total soil N values were highly significant ($P<0.001$, Table 3.4) between the eight sites and ranged from 0.21% in site 7 to 0.68% in site 2. There was also a highly significant difference ($P<0.001$) in two plant species, which the soil total N in perennial lupin stands always higher than pasture at same soil depth in each site. Further, significant effects were apparent for site by species ($P<0.001$), species by depth ($P<0.001$) and site by species by depth interactions ($P<0.001$), and in contrast there was a significant ($P<0.05$) site by depth interaction.

Total soil C showed large differences ($P<0.001$) between the eight sites and varied from 2.15% at site 7 to 8.46% at site 2. Total soil C in perennial lupin soils was higher than in neighbouring pasture soils. Total soil C varied ($P<0.001$) between perennial lupin and long-term established pasture land (Table 3.4). Total soil C ranged from 2.29% for B depth to 8.46% for A depth in perennial lupin. Total soil C in pasture land ranged from 2.15% for B depth to 6.63% for A depth. At the same site in same species, total soil N and total soil C also indicated strong differences ($P<0.001$) between soil depth that the values in A depth were always higher than in B depth. In perennial lupin site 2, total soil C values in A depth was nearly double that in B depth. The highly significant differences were observed in site by species ($P<0.001$), species by depth ($P<0.001$) and site by species by depth interactions ($P<0.001$), while there a significant ($P<0.05$) site by depth interaction.

3.3.2 Soil mineral N

Soil mineral N was different ($P<0.001$, Table 3.5) between eight sites, with a range of 3.7-59.5 kg/ha. The strong difference was observed in soil mineral N between perennial lupin and pasture, which the maximum value for lupin (59.5 kg/ha) was double that of pasture soil (24.5 kg/ha). Soil mineral N also was also strongly ($P<0.001$) affected by depth, that the value for A depth was always higher than for B depth in the same site for both two plant species. The mineral N was found to be significantly ($P<0.001$) different for site by species ($P<0.001$), species by depth ($P<0.001$) and site by species by depth interactions ($P<0.001$). Unlike soil mineral N significant ($P<0.05$) interaction effects were observed for site by depth interaction.

3.3.3 The relationship between forage type and soil N

For soil mineralizable N, the values differed strongly ($P<0.001$, Table 3.5 and Figure 3.3) between the eight sites, with values ranging from site 4 (18.8 kg/ha) to site 2 (213.2 kg/ha). A strong species

difference (P<0.001) in soil mineralizable N was observed between different forage types. The values for lupin were generally higher than for pasture for both soil profile at each site. The values for soil mineralizable N were also affected by soil depth (P<0.001), where A depth always had higher values than B depth. There were highly significant differences for site by species (P<0.001), species by depth (P<0.001) and site by species by depth interactions (P<0.001). The mineralizable N showed the highest values in lupin at site 2 at A depth and the lowest value showed in pasture land at site 4 with B depth. The interaction for site by depth showed significant (P<0.01) influence to soil mineralizable N (Table 3.5).

Table 3.4: Mean total soil N and total soil C concentrations analysed for perennial lupin and pasture at two depth at eight sites.

Site	Species	Depth +	Mean total soil N (%)	Mean total soil C (%)
1	Lupin	A	0.63	7.53
		B	0.37	4.20
	Pasture	A	0.55	6.63
		B	0.36	3.88
2	Lupin	A	0.68	8.46
		B	0.42	4.64
	Pasture	A	0.55	6.63
		B	0.36	3.88
3	Lupin	A	0.44	5.28
		B	0.31	3.34
	Pasture	A	0.40	4.01
		B	0.25	2.40
4	Lupin	A	0.46	5.45
		B	0.34	3.69
	Pasture	A	0.40	4.01
		B	0.25	2.40
5	Lupin	A	0.32	3.39
		B	0.26	2.68
	Pasture	A	0.30	3.84
		B	0.24	2.68
6	Lupin	A	0.26	2.77
	Pasture	A	0.23	2.53
7	Lupin	A	0.51	6.43
		B	0.39	4.76
	Pasture	A	0.21	2.25
		B	0.21	2.15
8	Lupin	A	0.21	2.35
		B	0.21	2.29
	Pasture	A	0.48	5.89
		B	0.35	4.30
	Mean		0.36	4.16
	Species	SEM	0.005	0.053
		LSD (5%)	0.010	0.104
	Depth	SEM	0.005	0.037
		LSD (5%)	0.010	0.037
P	Site		***	***
	Species		***	***
	Depth		***	***
	Site x Species		***	***
	Species x Depth		***	***
	Site x Depth		*	*
	Site x Species x Depth		***	***

***Significant at P< 0.001 level, *Significant at P< 0.05 level.

+: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.

Table 3.5: Mean soil mineral N and mineralizable N concentrations analysed for perennial lupin and pasture at two depth at eight sites.

Site	Species	Depth +	Mean mineral N (kg/ha)	Mean mineralizable N (kg/ha)
1	Lupin	A	11.9	172.2
		B	3.6	43.1
	Pasture	A	19.1	130.1
		B	8.0	38.7
2	Lupin	A	18.3	213.8
		B	4.8	56.1
	Pasture	A	19.1	130.1
		B	8.0	38.7
3	Lupin	A	24.6	87.1
		B	10.2	39.9
	Pasture	A	24.5	85.7
		B	5.7	18.8
4	Lupin	A	17.6	103.1
		B	11.0	48.0
	Pasture	A	22.0	85.7
		B	5.7	18.8
5	Lupin	A	21.6	90.8
		B	15.4	54.9
	Pasture	A	5.9	73.9
		B	4.9	46.9
6	Lupin	A	10.5	50.9
	Pasture	A	5.3	37.0
7	Lupin	A	59.5	165.6
		B	21.2	98.4
	Pasture	A	16.5	166.2
		B	15.3	91.1
8	Lupin	A	10.1	36.9
		B	9.1	30.2
	Pasture	A	10.8	39.9
		B	8.5	32.3
	Mean		14.3	77.5
	Species	SEM	0.60	2.22
		LSD (5%)	1.19	4.42
	Depth	SEM	0.59	2.21
		LSD (5%)	1.18	4.40
P	Site		***	***
	Species		***	***
	Depth		***	***
	Site x Species		***	***
	Species x Depth		***	***
	Site x Depth		*	**
	Site x Species x Depth		***	***

***Significant at P< 0.001 level, ** Significant at P< 0.01 level, *Significant at P< 0.05 level.

+: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.

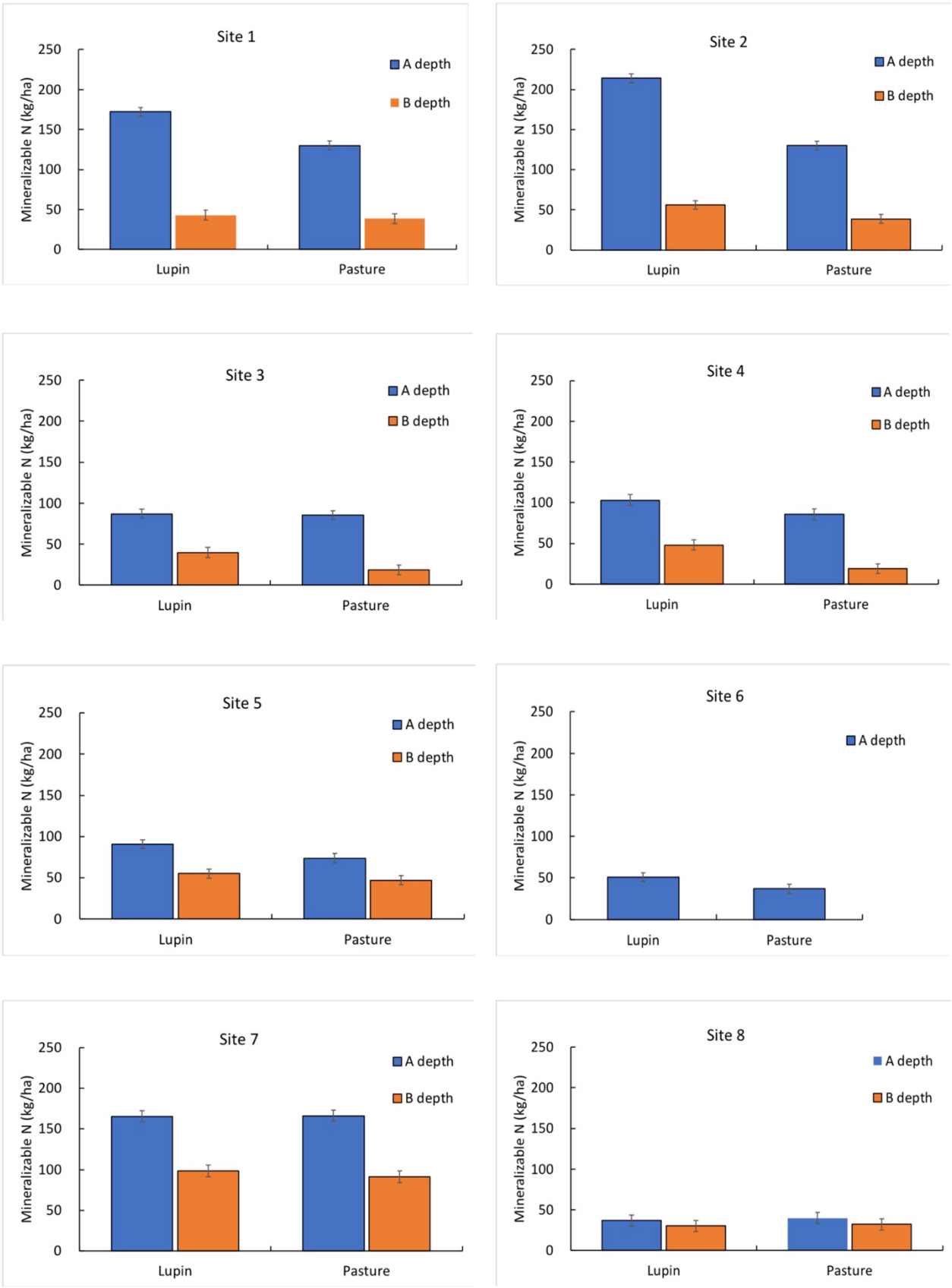


Figure 3.3: Mean soil mineralizable N analysed for perennial lupin and pasture at two depths at eight sites. Soil depth 'A' = 0-7.5 cm horizon, soil depth 'B' = 7.5-15 cm horizon. Lines on bars represent one SEM.

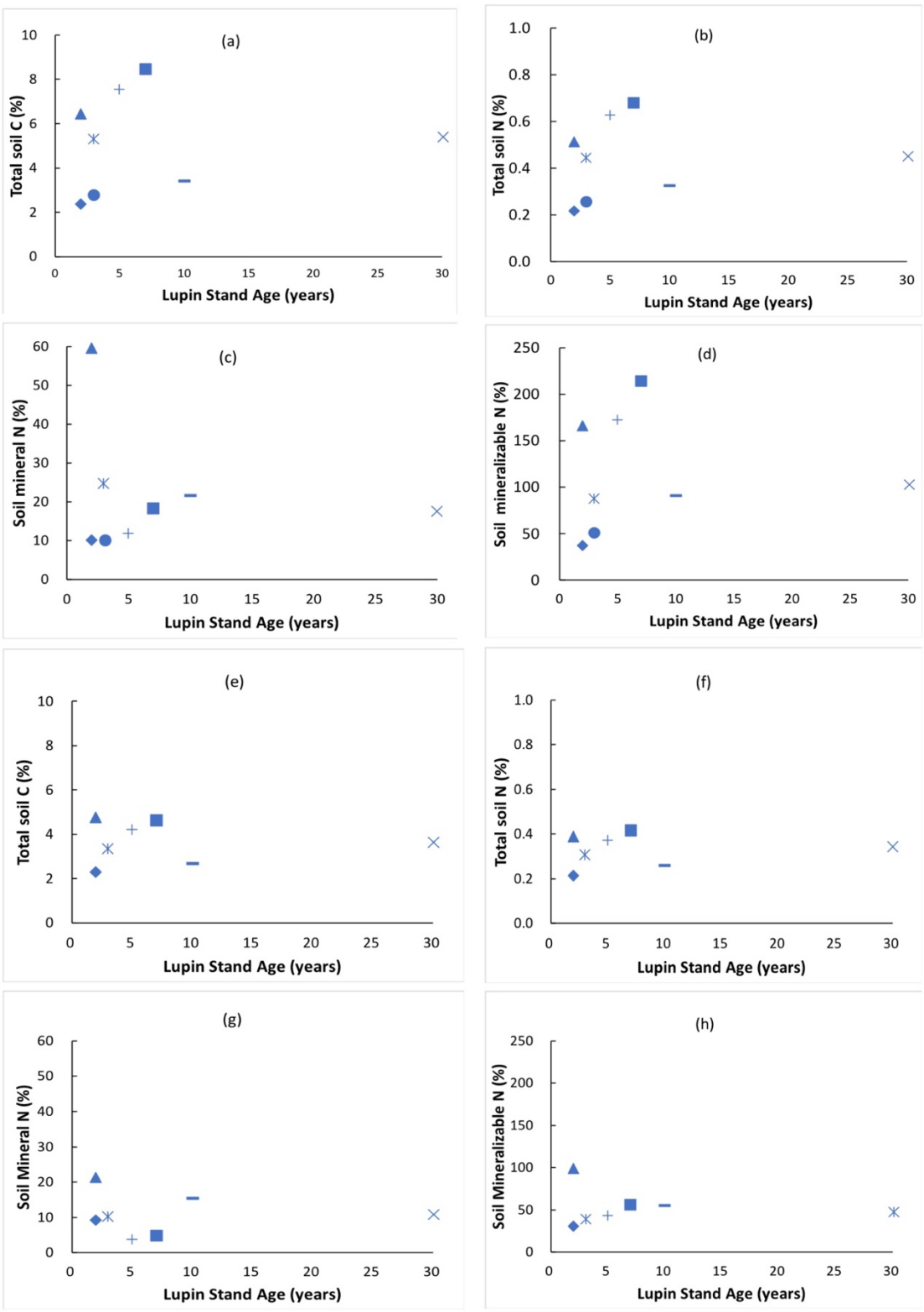


Figure 3.4: The relationship between mean total soil C, total soil N, soil mineral N and soil mineralizable N concentration analysed by different ages of perennial lupin stands at eight field sites in soil depth 'A' = 0-7.5 cm horizon (a,b,c,d) and soil depth 'B' = 7.5-15 cm horizon (e,f,g,h). Site 1: (+), site 2: (▪), site 3:(✳), site 4:(×), site 5:(–), site 6:(●), site 7:(▲), site 8:(◆).

3.3.4 Temporal relationship between the age of lupin stand and soil N level

Soil mineral N of perennial lupin soils was significant (P<0.001) affected by different soil depths, with the values in A depth always higher than B depth (Figure 3.4). In site 2, the large difference was observed with 18.32 kg/ha for A depth which was more than the 4 times for B depth (4.83 kg/ha). Lupin stand age also highly significant (P<0.001) affects on soil mineral N, that range from 3.65 kg/ha to 59.45 kg/ha between lupin stand ages. The lupin stand age by depth interaction showed no significant effect for soil mineral N (Table 3.6). Soil mineralizable N values are significantly affected by soil depth, which always showed higher values in A depth (Figure 3.4). The highest soil mineralizable N value occurred in site 2 by A depth (213.8 kg/ha) which was nearly 4 times higher than B depth (56.1 kg/ha) in the same site and much higher than the mean value (86.1 kg/ha) among the 8 sites. At the same time, there was a significant (P<0.001) difference in soil mineralizable N affected by lupin stand age. The interaction of soil depth and lupin stand age indicated the significant difference (P<0.001) in soil mineralizable N (Table 3.6).

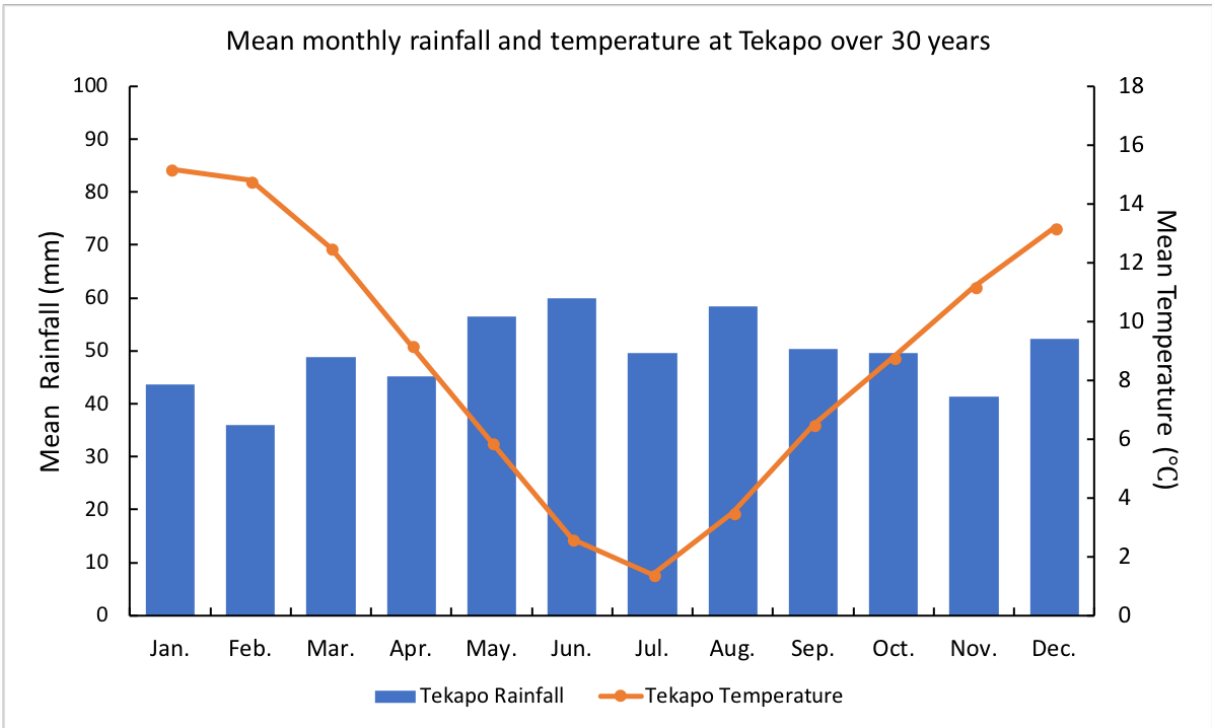
At A depth, the total soil N and total soil C values increased by increasing perennial lupin age in site 1,2,6 and 8 (Figure 3.4a, b). However, in site 7 and site 3, total soil N and total soil C showed higher values than site 8 and site 6 with the same lupin age. Total soil N and C showed intermediate values which are similar to the mean value of site 5 and site 4 with 10 and 30 years lupin, respectively. In site 1,2,5,6 and 8, the values of soil mineral N increased by increasing lupin ages. Site 7 and site 3 showed higher soil mineral N values compared with site 8 and site 6. (Figure 3.4c). Soil mineralizable N values generally increased by increasing lupin ages in site 1,2,6 and 8. Compared with site 8 and site 6, soil mineralizable N showed higher values in site 7 and site 3 with the same lupin age. Soil mineral N and soil mineralizable N in site 4 showed meditate values compared with other sites (Figure 3.4c, d).

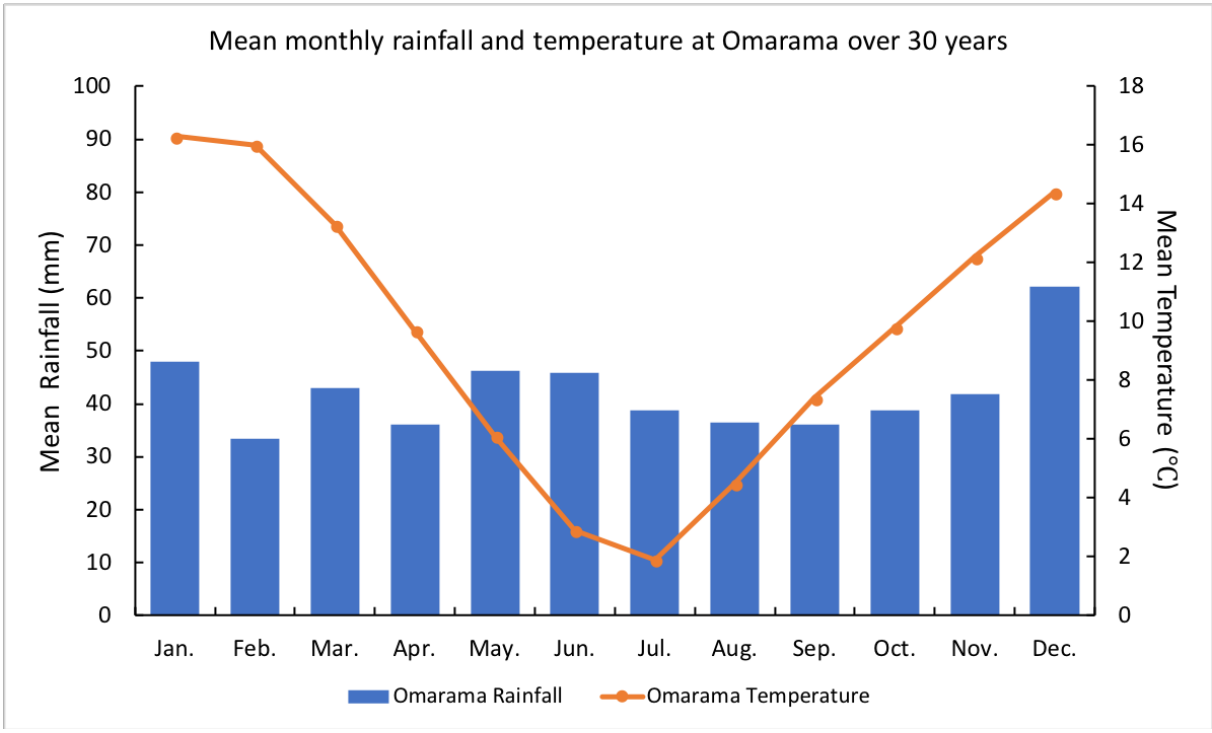
At B depth, total soil N and C showed the similar trend as A depth, that the values of total soil N and C generally increased with increasing lupin age, and site 7 showed higher values than site 8 with the same lupin age. Site 5 and site 4 showed mediate values closed to mean values in total soil N and C (Figure 3.4e, f). Soil mineral N values in B depth changed irregularly with increasing lupin age (Figure 3.4g). However, site 1, 2, 3 and 8 showed increasing soil mineralizable N by increasingly lupin age. Site 4 and site 5 showed the decrease in soil mineralizable N by lupin age (Figure 3.4h)

Table 3.6 Mean total soil N, total soil C, mineral N and mineralizable N analysed from lupin soils in eight sites with 2 depths by different lupin ages.

Site	Site Age (Year)	Depth +	Mean total soil N (%)	Mean total soil C (%)	Mean mineral N (kg/ha)	Mean mineralizable N (kg/ha)
1	5	A	0.63	7.53	11.9	172.2
		B	0.37	4.20	3.6	43.1
2	7	A	0.68	8.46	18.3	213.8
		B	0.42	4.64	4.8	56.1
3	3	A	0.44	5.28	24.6	87.1
		B	0.31	3.34	10.2	39.9
4	30	A	0.46	5.45	17.6	103.1
		B	0.34	3.69	11.0	48.0
5	10	A	0.32	3.39	21.6	90.8
		B	0.26	2.68	15.4	54.9
6	3	A	0.26	2.77	10.5	50.9
7	2	A	0.51	6.43	59.5	165.6
		B	0.39	4.76	21.2	98.4
8	2	A	0.22	2.35	10.1	36.9
		B	0.21	2.29	9.1	30.2
	Mean		0.39	4.48	16.6	86.1
	Depth	SEM	0.020	0.273	2.63	7.86
		LSD (5%)	0.041	0.549	5.29	15.80
	Site Age	SEM	0.036	0.480	4.63	13.82
		LSD (5%)	0.082	0.966	9.31	27.80
	P	Depth	***	***	***	***
		Site Age	***	***	**	***
		Depth x Site Age	**	**	ns	***

***Significant at P< 0.001 level, ** Significant at P< 0.01 level, ns - no significant difference.
+: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.





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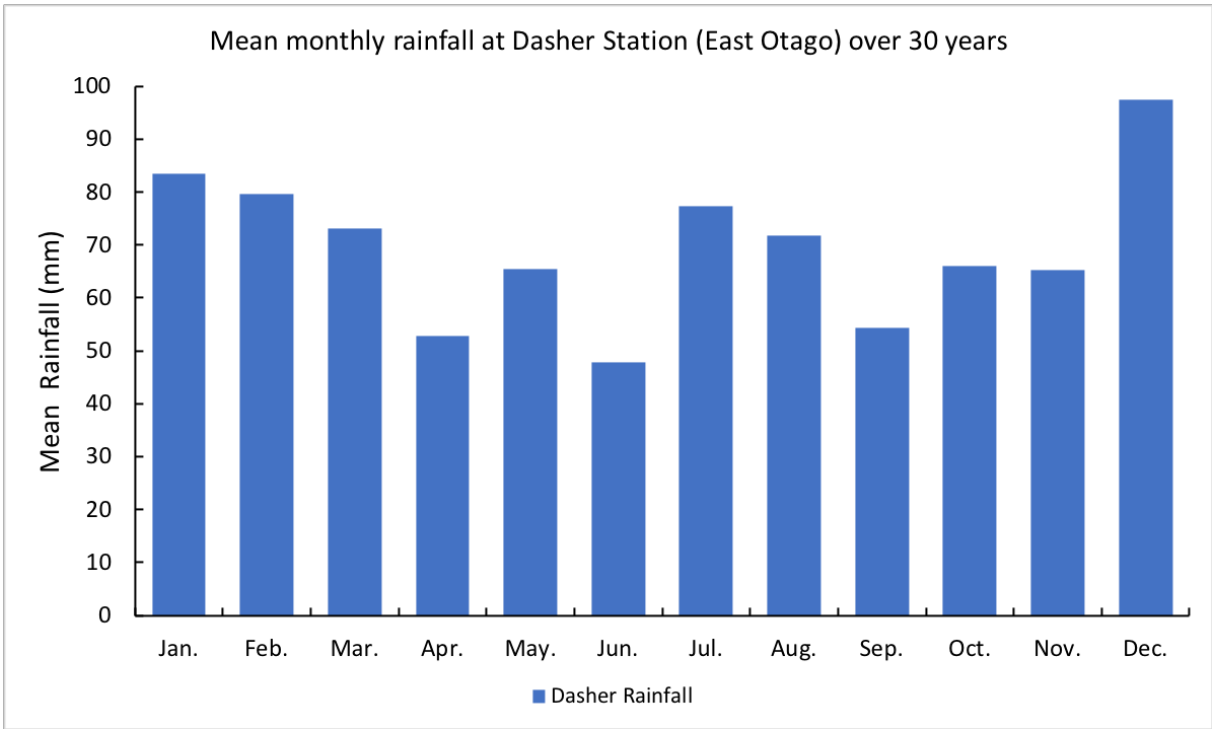

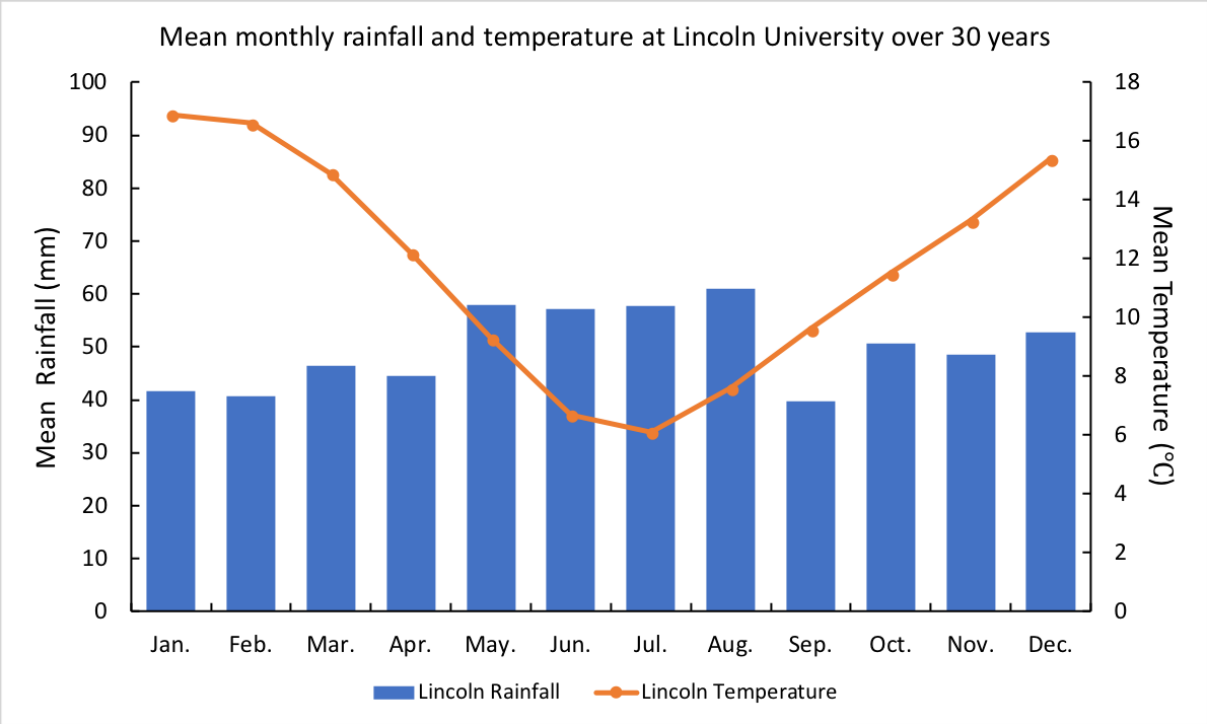

Figure 3.5 Mean rainfall and temperature collected over 30 years at different sites. (The figure showed rainfall of Dasher Station only due to no record for temperature for Dasher Station).

3.4 Discussion

3.4.1 Soil mineral N and soil mineralizable N

Soil mineralizable N varied dramatically between the eight sites which was caused by forage species, different soil conditions, annual rainfall levels and the age of lupin**.** The highest value occurred in site 2 with 213.8 kg/ha with nearly seven times higher than site 8. The values of soil mineralizable N differed strongly between soil depths. A depth always had higher N values and the differences between two depths varied between sites. We have known about the trend of soil N changes by soil depths in long-term pasture soils, while we have no idea about the soil N content for perennial lupin soils. For example, Hossain *et al*. (1996) showed a similar trend of soil mineralizable N by soil depth that subsurface soil samples had lower values than the surface soil by the same incubation treatment. However, we found some new and important results from the field study, that soil mineralizable N also decreased by increasing soil depth from perennial lupin soils.

A significant difference in soil mineralizable N also occurred between perennial lupin and pasture soils. Generally, lupin soils had higher values than pasture, especially in site 1 and site 2. Waring and Gibson (1994) and Hossain *et al*. (1996) illustrated the same changes of plant available soil N after introducing the legumes into the pasture. The higher soil mineralizable N occurred in legume-based soils than original pasture or cropping soils.

However, site 8 showed higher soil mineralizable N in pasture soils at both depths and site 7 showed higher pasture soil mineralizable N in the A depth. Site 8 also showed a different result compared to the other seven sites with total soil C and N which can be explained by the conditions at the site. Site 8 was Lincoln University, located in Christchurch with lower altitude, higher annual average temperature and rainfall (Figure 3.5) and lower soil acidity (Table 3.3) than other high country sites, results in less chilling stress, less drought stress and less Al toxicity for both legume and pasture land. The lupin stand at Lincoln University was actually mixed with a low abundance of lupin and therefore a high abundance of cocksfoot. The pasture plot at this site was dominated by perennial ryegrass. The low plant density of lupin at this site could have resulted in lower total soil C and soil N accumulation than normal pasture with less growth stress for perennial ryegrass, which may lead to higher total soil N and C in pasture soils.

The strong significant difference of soil mineral N between eight sites was expected in this study. The large differences between of N fixation in pasture were likely influenced by climate conditions such as temperature and rainfall during the perennial legumes growth varied considerably between years (Carlsson & Huss-Danell, 2003). The variations of soil conditions between different sites such as soil pH and soil fertility also resulted in a great difference of soil mineral N of different sites.

Soil mineral N was also significantly different between soil depths, where soil mineral N decreased with increasing soil depth. This result had the showed the same trend as Xu *et al*. (1996) where topsoil depth accounted much higher soil mineral N than sub-soil depth under different land use. The higher values of soil mineral N with A depth was caused by accumulation of additional N-rich plant residues and root nodule sourced N in the topsoil from the N-fixing perennial lupin. Furthermore, the highly significant influence on soil mineral N by different forage types observed in each site. Lupin soils had higher values than established pasture soils, in site 3, 5, 6, and 7 while pasture land had higher values of soil mineral N in site 1, 2, 4 and 8. Soil mineralization process could be affected by climate, microorganisms and plant residues, which were highly variable to release mineral N into the soil (Jarvis *et al*., 1996). Although soil mineral content was small, it can turn over and maintain plant-available N requirements over several days. Thus, some mineralization could already occur by the time that soil samples were taken. It also has been reported that soil mineral N was very dynamic even over very short period (Dividson *et al*., 1990). However, soil mineralizable N are more reliable, which could show soil N status better than soil mineral N.

3.4.2 Temporal relationship between lupin age and soil N status

In previous sections, it was reported that values of total soil C, total soil N, mineral N and soil mineralizable N between eight sites could result from different soil conditions, climates and plant density and ages of the perennial lupin stands. In this section, the relationship between lupin age and soil N level is discussed. The result in section 3.2.5 demonstrated significant differences between A depth and B depth in total soil C, total soil N, mineral N and soil mineralizable N. The higher plant residues in A depth soils lead to the higher values in A depth in total soil C, total soil N, soil mineral N and soil mineralizable N. Table 3.3 indicated that mean total soil N and total soil C both have strongly significant differences between different site ages. Sites 1 to 5 were close to Lake Tekapo where the site 1, 2 and 3 had the same pasture soil to compare to. At sites 1, 2 and 3, it was found that mean total soil N and total soil N values increased by increasing lupin age. Site 3 had the lowest values with youngest lupin age and site 2 had highest values with oldest lupin age. The same trend also demonstrated in mean soil mineralizable N, illustrating higher soil mineralizable N content where the lupin stand was older. These results strongly indicate soil N accumulation with increasing age of the lupin stand.

However, the converse result was seen for mean soil mineral N. The oldest lupin soils indicated lowest soil mineral N which was available for plant take up. Soil mineral N could be converted from organic forms of N by organic matter decomposed by microorganisms. Soil mineral N is extremely dynamic, which could be changed by time and space. The soil mineralization affected by soil conditions such as moisture, temperature and soil microorganisms (Cassman & Munns, 1980). It has

been reported that some mineralization had already occurred by the time when soil samples were taken from sites (Jarvis *et al*., 1996). In this study, soil samples were analysed 3-5 days later after soil sampling, which could have got the completely different of soil mineral N. The large differences between soil mineral N and soil mineralizable N can be regarded as the differences between soil mineral N and soil organic N. Jarvis *et al*. (1996) also indicated the large differences that the mineral N pool was always much smaller than organic N pool. That study also illustrated the highly variable of soil mineral N in spatial.

For site 4 and 5, although they were also located near Lake Tekapo, the values of those two sites did not show higher values than other younger stand of lupin. While there were also similar changes only between those two sites. The soils for the older lupin stand indicated higher values of mean total soil C, total soil N and soil mineralizable N and the lower soil mineral N value. The reason why sites 4 and 5 indicated the lower values than other sites in Tekapo with higher lupin ages was possibly a lower number of rhizobia in the soil or the poor symbiotic effectiveness of those rhizobia (Ballard *et al*., 2003). However, this reason should be tested in future experiments. Additionally, different fertiliser application history (Table 3.1) may result in the difference which the low and nil fertiliser application with site 4 and site 5 respectively, compared with higher fertiliser application at sites 1,2 and 3 lead to the lower values in soil N level.

Site 6, 7 and 8 were Omarama Station, Dasher Station and Lincoln University, respectively. Soil condition at site 6 in Omarama was very stony, which could inhibit large root system development of perennial lupin and the ability of the soil store N. Although for lupin stand under 3 years of age, the values of each part were lower than the site with the same lupin age (site 3). In contrast to site 6, site 7 with two years lupin age demonstrated higher values. The higher values of site 6 were mean resulted from higher rainfall (Figure 3.5) in Otago, which made appropriate soil moisture for plant growth. Such low values of site 8 in Lincoln University may result from low lupin density, high annual temperature and low altitude for Canterbury plains. The young age of lupin stands might lead to the low values in total soil C total soil N and soil mineralizable N. The high plant density of grass in Lincoln University experiment area resulted in the decrease in soil N from a lot of N uptake by grass.

3.4.3 Total soil N and C

The highly significant influence of soil depths on total soil N was apparent for both forage types. The input of plant residues on topsoil lead to higher values of total soil N always occurred in A depth (Jackman, 1964). Watson (1963) and Vallis (1972) reported that legumes could increase soil N which lead to higher soil fertility and increased production with aboveground and belowground, while they didn't refer the soil N trend for lupin stands. In this study, total soil N showed highly significant difference between perennial lupin, and pasture. The lupin soils resulted in higher total soil N than

pasture soils in both depth which corresponds the report by Watson (1963) and Vallis (1972). Although these results provide some evidence of soil N accumulation with increasing age of lupin stand, further research incorporating more sites is required to confirm any temporal influences.

There was a highly significant difference in total soil C between different sites. Post *et al*. (1982) reported that the amount of soil C was strongly correlated annual rainfall. Similarly, Adams (1980) reported that total soil organic C content increased with precipitation and decreased with temperature. Total soil C differences between the sites in this study were expected because soil conditions, climates and plant species differed. Site 2 A depth showed the highest value of total soil C followed by site 1 A depth, which were both growing perennial lupin. Soil depth also had a highly significant effect on total soil C with A depth always having higher values than B depth, both in perennial lupin and pasture soils. The higher total soil C with A depth was to be expected as that soil carbon was strongly controlled by soil depth and climate influence at the shallow layers (Jobbágy & Jackson, 2000). There is a marked decline in soil organic C with depth (Arrouays & Pelissier, 1994; Bernoux *et al.*, 1998), because carbon return to the soil is via senescence of above ground herbage and roots which have more influence at the surface soil layer.

Soil total C showed significant difference between the forage types. The result that perennial lupin soils generally had higher total soil C than pasture soils was expected. It resulted from the decreased soil organic C in long-term pasture land (Schipper *et al*., 2007) and the increased soil C content by legume (Conant *et al*., 2001). It has been reported that introducing legumes into pasture systems often results in increased forage biomass production, including below-ground (Conant *et al*., 2001; Crawford *et al*., 1996; Robinson & Jacques, 1958), which contributes to in increased soil C.

In this field study, there was a limitation in assessing changes in total C + N due to there being a small number of sites with different ages of lupin stands as well as a number of the sites, being for a short duration (i.e. < 5 years). This makes it difficult to determine whether differences are due to age, climate, soil type or initial soil levels.

3.5 Conclusions

The objective of the research was to determine the effects of perennial lupin stands of varying ages on N concentrations in acid high country soils. In this study, total soil C, total soil N, soil mineral N and soil mineralizable N have been analysed in the plant rooting zone to 15cm of acid high country soils and a fertile lowland soil across eight different sites.

Soil mineral N and mineralizable N demonstrated similar trends as total soil C and N. Soil mineral N and mineralizable N where affected by site-specific climate and soil conditions. Different forage types influenced soil mineral N and mineralizable N, which lupin soils in general having higher values than

pasture soils. Soil A depth lead to higher mineralizable N than B depth. The highest value of soil mineralizable N occurred at site 2 for the A depth of the lupin soil with 213.8 kg/ha.

For both lupin soils and pasture soils, total soil N and C, soil mineral N and soil mineralizable N varied across the eight sites. The two soil depths differed in total soil N and C, soil mineral N and soil mineralizable N, where A depth having higher values than B depth. Lupin age affected soil N level. Generally, total soil N and soil mineralizable N increased with increased age of the lupin stands.

Total soil C and N differed across the eight sites, which were likely influenced by interactions of climate. The maximum value of total soil N and total soil C occurred in site 2 with 0.68% and 8.5% at A depth in the lupins soils. The two soil depths were different in total soil N and C. Increasing soil depth showed a decline in total soil C and N, and the values for A depth being nearly double than those at B depth. Total soil C and N was also affected by different forage types, with the perennial lupin soil having higher total soil C and N than long-term established pastures soils.

Chapter 4

Glasshouse Experiment

4.1 Introduction

The glasshouse experiment was necessary to quantify more accurately the effect of perennial lupin on the amount of plant-extractable mineralizable N in these acid high country soils. The experiment used a nutrient exhaustion method whereby annual ryegrass (*Lolium multiflorum*) grew in the soil until it exhausted the supply of plant-available N.

4.2 Material and Method

4.2.1 Soil collection

Bulk soil samples were collected from five sites, each with an established stand of perennial lupin and an adjacent pasture, on three high country farms in the South Island: Glenmore station (site 1), Glenmore Station Original (site 2), Glenmore Station New experiment area (site 3), Sawdon Station (site 4) and Omarama Station (site 5). Soil from each lupin stand and pasture at each site was sampled at two depths (0-7.5 cm (A) and 7.5 cm (B) horizons) with six replications for each. The soil condition at Omarama Station was stoney, so we only sampled A depth with both lupins stand soils and pasture soils for Omarama site, which was the same as the field study.

Plate 4.1: Soil collecting for the glasshouse experiment on 8 November 2017 in summer, Sawdon Station (site 5). Photo credit: Jim Moir.

The soil samples were collected on 8 November 2017 and transported back to Lincoln University. The collection process involved digging small sods with a spade for each forage type at each site. Soil analyses were done before plant establishment of the glasshouse experiment.

4.2.2 Glasshouse conditions

The experiment was located in the Aluminex Glasshouse at Lincoln University Nursery on Farm Road. The temperature of glasshouse was monitored by a sensor. A data logger with a thermocouple sensor positioned 2m above ground monitored the air temperature in the glasshouse. A computer programme adjusted the air temperature to avoid extreme high and low temperatures. When the temperature was below 16°C, hot water pipes along the walls heated the glasshouse. When the temperature was above 24°C, a fan at the northern end of the glasshouse ventilated the warm air out and a fan at the southern end of the glasshouse drew air in through wet pads to cool the air in the glasshouse.

4.2.3 Fertiliser application

Fertilisers were mixed into the soil samples to help the annual ryegrass grow better and therefore extract the N sufficiently. Phosphorus, K, S and lime were applied at the following rates: $Ca(H_2PO_4)_2.H_2O$ (MCP) was applied as 200 mg P/L, K_2SO_4 was applied as 100 kg S/ha and Lime ($CaCO_3$) was added to change the soil acidity as 2000 kg $CaCO_3$/ha. After calculation, the fertilisers were weighed separately as 0.2 g $Ca(H_2PO_4)_2.H_2O$ (MCP), 0.22 g K_2SO_4, and 0.66 g $CaCO_3$ in small vials respectively for each pot.

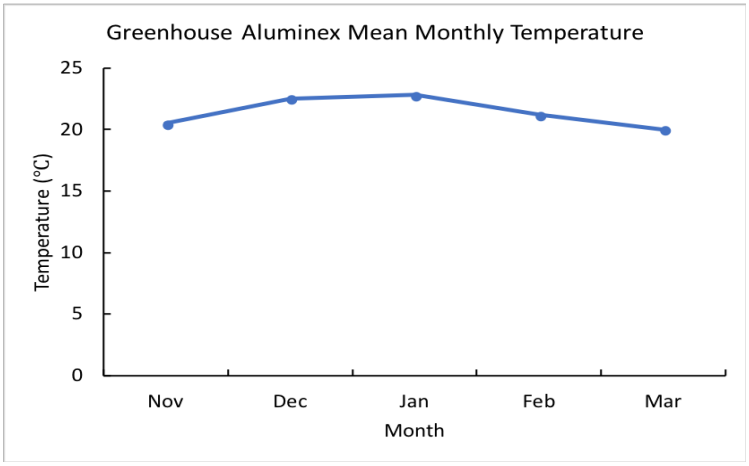

Figure 4.1 Air temperature regime experienced by the annual ryegrass during the glasshouse experiment in the Aluminex Glasshouse at Lincoln University Nursery.

Plate 4.2: The glasshouse experiment was located in the Aluminex Glasshouse at Lincoln University. Photo Credit: Xueying Che.

4.2.4 Plant establishment

Potting and seeding took place on 16 November 2017. The experiment tested the soil from each of the five sites at each of the two depths in a completely randomised block design with six replications. The lupin soils of site 1, site 2 and site 3 shared same pasture soil to compare with at Glenmore Station, while soils from lupins site 4 and 5 compared with their own pasture soils to determine the soil N content. Eighty-four pots of soil were established in total.

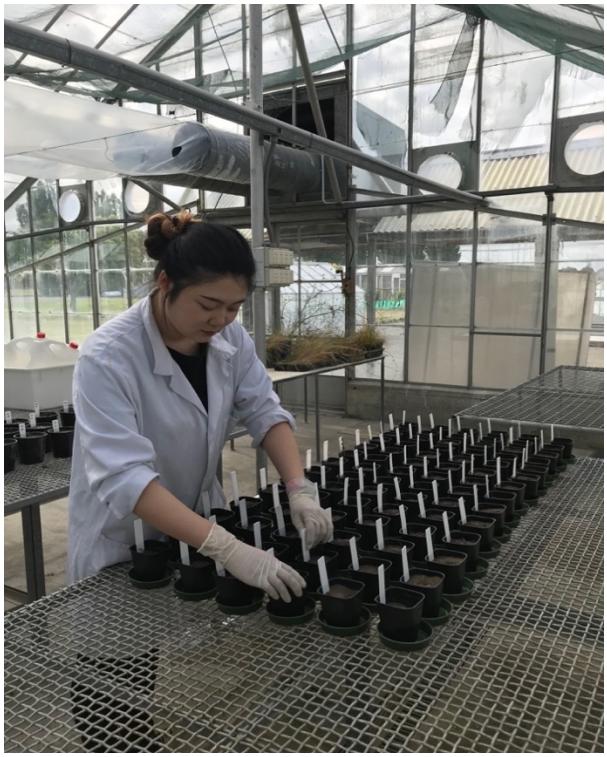

Plate 4.3: Plant establishment on 16 November 2017. Photo Credit: Kevin Zhou.

The potting procedure filled each pot with soil and then put the soil into a clean plastic bag. It then added some soil from the bag to a vial of the first fertiliser, finely mixed the soil and fertiliser in the vial and then put it back into the plastic bag, finely remixing the soil again in the bag. Then, the procedure added another fertiliser until all three applications of fertiliser finished. It then put all the mixture back into each pot and placed it in a clean saucer under each pot. The pots were 275 ml volume each and with 245 ml of soil in each pot. The annual ryegrass seeds were sown at ten seeds per pot, planted at 1-2 mm depth from topsoil, covered with a thin layer of soil sown in each pot. After ten days, all pots were thinned to a final plant density of five plants per pot. Watering each pot every morning with about 12-15 ml deionised water. During especially high temperature occurred, the pots were watered two times a day, morning and dusk. Watering allowed enough soil moisture to cope with the high quality of plant growth while avoiding nutrient leaching and waterlogging each time.

4.2.5 Harvest

The ryegrass was cut to 3 cm above the surface soil level at each harvest. Harvests occurred nearly every 3 weeks and continued for approximately 5 months to the final harvest. All harvest samples from each plot were collected, stored and analysed for and shoot yield. A total of six harvests were performed on 12 December, 27 December in 2017 and 13 January, 3 February, 6 March and 28 March in 2018. In this exhaustive harvesting system, the content of shoot N was collected and be measured after the final harvest.

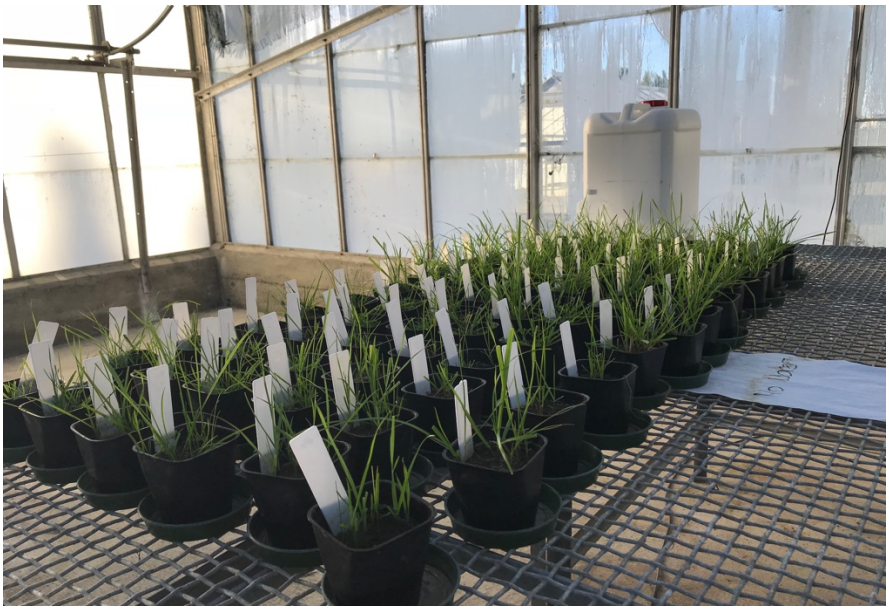

Plate 4.4: Annual ryegrass plants in the glasshouse experiment before the first harvest on 11 December 2017. Photo Credit: Xueying Che.

4.2.6 Measurements

Once harvested, all herbage samples were weighed for fresh weight, then oven dried at 70°C for 48 hours, weighed and finely ground. Samples were bulked on a pot basis, then were analysed by NIR machine (McGregor Muffle. W. D. McGregor Ltd, model LAB 3A, KW 5000W, Max T:1000c) for calculating plant N concentration. The N uptake by the ryegrass was calculated as plant dry weight multiplied by plant N concentration.

4.2.7 Soil chemical analysis

All soil samples were analysed for soil mineral N, mineralizable N through the method Keeney and Bremner (1966) before plant establishment, and the dry plant materials were analysed using a near infrared spectroscopy (NIRS) machine.

Table 4.1 The methods of analyses used in glasshouse experiment.

Analysis	Method
Soil mineral N and mineralizable N	Keeney and Bremner (1966)
Herbage	NIR machine

4.2.8 Statistical analysis

All data sets were analysed to test for treatment effects by conducting an analysis of variance (ANOVA) using GENSTAT 16 (Lawes Agricultural Trust, Rothamsted, UK). For the glasshouse experiment, the treatment effects analysed by ANOVA were shoot DM yields, shoot N concentrations, N uptake per pot, soil mineral N and soil mineralizable N.

4.3 Results

4.3.1 Soil mineralizable N and soil mineral N

There were highly significant ($P<0.001$) differences between soil mineralizable N concentration between different sites, ranging from 34.9 to 205.1 kg/ha (Table 4.2). Forage type had highly significant ($P<0.001$) effect on soil mineralizable N. Generally, lupin soils had higher soil mineralizable N than pasture soils. Soil mineralizable N also significantly affected by site by species ($P<0.001$), site by depth ($P<0.001$), species by depth ($P<0.001$) and site by species by depth ($P<0.001$) interactions.

A highly significant ($P<0.001$) difference in soil mineral N was observed between sites (Table 4.2). Plant species also lead to strongly significant ($P<0.001$) differences in soil mineral N. Lupin soil had higher soil mineral N values than pasture. The interaction of species by depth indicated a significant

(P<0.01) influence on soil mineral N. The interactions of site by species and sites by species by depth showed no significant influence on soil mineral N level. Soil mineralizable N and soil mineral N values were also significantly (P<0.001) influenced by soil depths. The values of soil mineralizable N with a depth were higher for A than B depth, especially from lupin soils.

4.3.2 Dry matter yield

There were highly significant (P< 0.001) differences in the (DM yields between soils from the five sites, ranging from 0.36 to 0.75 g/pot (Table 4.3; Figure 4.3). A highly significant (P< 0.001) difference was also observed between different forage species soils. In the same site, ryegrass DM yield showed higher values in perennial lupin than in pasture soils. The DM yield values were also significantly (P< 0.001) affected by soil depth. The values of DM yield for soil from the A depth were always higher than soil from the B depth. Furthermore, highly significant effects were also observed for site by species (P<0.001), species by depth (P<0.001) and site by depth interactions (P<0.001) (Table 4.3). There was no significant site by species by depth interaction.

Table 4.2: Mean mineral N and mineralizable N of soil analysed from two depths in a perennial lupin stand and an adjacent pasture at each of five high country sites in the South Island.

Site	Species	Depth +	Mean mineral N (kg/ha)	Mean mineralizable N (kg/ha)
1	Lupin	A	19.8	162.7
		B	7.2	47.8
	Pasture	A	4.0	35.4
		B	7.9	43.5
2	Lupin	A	19.3	205.1
		B	7.2	52.6
	Pasture	A	4.0	35.4
		B	7.9	43.5
3	Lupin	A	12.0	111.5
		B	7.0	55.1
	Pasture	A	4.0	35.4
		B	7.9	43.5
4	Lupin	A	11.0	69.2
		B	12.6	54.8
	Pasture	A	6.6	47.8
		B	7.1	34.9
5	Lupin	A	11.8	50.3
	Pasture	A	5.3	36.4
	Mean		9.0	64.7
	Species	SEM	2.03	1.11
		LSD (5%)	4.41	2.34
	Depth	SEM	1.30	1.11
		LSD (5%)	2.73	2.35
P	Site		***	***
	Species		***	***
	Depth		*	***
	Site*Species		ns	***
	Site*Depth		*	***
	Species*Depth		**	***
	Site*Species*Depth		ns	***

***Significant at P< 0.001 level, ** Significant at P< 0.01 level, *Significant at P< 0.05 level, ns - no significant difference. +: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.

Table 4.3: Mean dry matter yield, shoot N and N uptake of annual ryegrass grown in a glasshouse in soil collected and analysed from two depths in a perennial lupin stand and an adjacent pasture at each of five high country sites in the South Island.

Site	Species	Depth +	DM Yield (g/pot)	Shoot N (%)	N Uptake (mg N/pot)
1	Lupin	A	0.73	2.57	1.89
		B	0.45	2.46	1.10
	Pasture	A	0.54	2.60	1.41
		B	0.36	2.47	0.90
2	Lupin	A	0.75	2.79	2.09
		B	0.40	2.54	1.01
	Pasture	A	0.54	2.60	1.41
		B	0.36	2.47	0.90
3	Lupin	A	0.69	2.68	1.81
		B	0.43	2.71	1.17
	Pasture	A	0.54	2.60	1.41
		B	0.36	2.47	0.90
4	Lupin	A	0.63	2.70	1.69
		B	0.39	2.52	0.96
	Pasture	A	0.42	1.91	0.81
		B	0.35	2.19	0.76
5	Lupin	A	0.54	2.39	1.29
	Pasture	A	0.39	2.27	0.89
	Mean		0.49	2.50	1.24
	Species	SEM	0.027	0.046	0.070
		LSD (5%)	0.054	0.092	0.139
	Depth	SEM	0.047	0.046	0.043
		LSD (5%)	0.034	0.034	0.086
P	Site		***	***	***
	Species		***	***	***
	Depth		***	***	***
	Site*Species		***	***	ns
	Site*Depth		***	***	*
	Species*Depth		***	***	***
	Site*Species*Depth		ns	**	ns

***Significant at P< 0.001 level, ** Significant at P< 0.01 level, *Significant at P< 0.05 level, ns - no significant difference. +: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.

4.3.3 Shoot N concentrations

Shoot N concentration was strongly affected (P< 0.001) by soil site (Table 4.3; Figure 4.3). The maximum value of shoot N concentration was observed in site 2 with A depth in perennial lupin soil (2.79%) and the minimum value occurred at site 4 with B depth in pasture soil. Shoot N concentration differed between the soils from various forage stand (P< 0.001). Generally, shoot N was higher values in perennial lupin soils than pasture soils. Soil sample depth significantly (P< 0.001) influenced shoot N concentration. The higher values of shoot N concentration were observed in A depth with about 0.12% higher than B depth. Site by species (P< 0.001), site by depth (P< 0.001) and species by depth (P< 0.001) interactions showed highly significant effects on shoot N values. The interaction of site by species by depth showed significant (P< 0.01) influence on ryegrass shoot N concentrations.

4.3.4 N uptake

There was a highly significant (P< 0.001) difference in the plant N uptake between the five soil sites, ranging from 0.76 to 2.09 mg N/pot (Table 4.3; Figure 4.2; Figure 4.3). Different forage species indicated the high significant (P< 0.001) effect on N uptake by ryegrass. N uptake by ryegrass showed higher values from perennial lupin soil than pasture soil. N uptake values were highly significantly (P<0.01) affected by soil depth. The soil from A depth always resulted in higher ryegrass N uptake mean values than the soil from B depth. In contrast, there was no significant influence on N uptake from site by species and site by species by depth interactions. The N uptake values per pot were significant (P<0.05) affected by the interaction of site by depth. The highly significant (P< 0.001) differences of mean N uptake also occurred in species by depth interaction.

4.3.5 Temporal relationship between the age of lupin and N uptake

Perennial lupin stands ages indicated highly significant (P< 0.001) effect on ryegrass DM yield (Table 4.4; Figure 4.3). The maximum DMY occurred in site 2 soil with 7 years old lupin. Soil depth also showed strongly significant effect on plant DM yield, that A depth indicated higher values than B depth. There was a highly significant (P< 0.001) difference of ryegrass DM yield observed in site age by depth interaction.

There was a significant (P<0.05) difference in mean shoot N concentrations between the five sites, ranging from 1.91 to 2.79 % soil sample. Depth indicated the significant influence on mean shoot N, that mean shoot N with A depth were always higher than B depth. However, the interaction of lupin stand age by depth showed no significant effect on mean shoot N (Table 4.4).

The age of lupin stand showed a highly significant (P< 0.001) effect on mean N uptake by ryegrass. The values of N uptake by plant were also highly significant (P< 0.001) influenced by soil depth, where A depth had higher values than B depth. At site 2 with 7 years lupin stand indicated more than double higher value with A depth than B depth. While there was no significant influence on mean N uptake by ryegrass from site age by depth interaction (Table 4.4; Figure 4.3).

Table 4.4: Mean shoot dry matter yield, shoot N concentration and N uptake of annual ryegrass grown in a glasshouse in soil collected and analysed from two depths in a perennial lupin stand at each of five high country sites in the South Island.

Site	Site Age (Year)	Depth +	DM Yield (g/pot)	Shoot N (%)	N Uptake (mg N/pot)
1	5	A	0.73	2.57	1.89
		B	0.45	2.46	1.10
2	7	A	0.75	2.79	2.09
		B	0.40	2.54	1.01
3	30	A	0.69	2.68	1.81
		B	0.43	2.71	1.17
4	10	A	0.63	2.70	1.69
		B	0.39	2.52	0.96
5	3	A	0.54	2.39	1.29
		Mean	0.56	2.59	1.44
	Site Age	SEM	0.044	0.876	0.109
		LSD (5%)	0.089	0.177	0.221
	Depth	SEM	0.027	0.054	0.068
		LSD (5%)	0.055	0.110	0.137
	p	Site Age	***	*	***
		Depth	***	*	***
		Site Age*Depth	***	ns	ns

***Significant at P< 0.001 level, * Significant at P< 0.01 level, ns - no significant difference

+: Soil depth 'A' = 0-7.5cm horizon, soil depth 'B' = 7.5-15cm horizon.

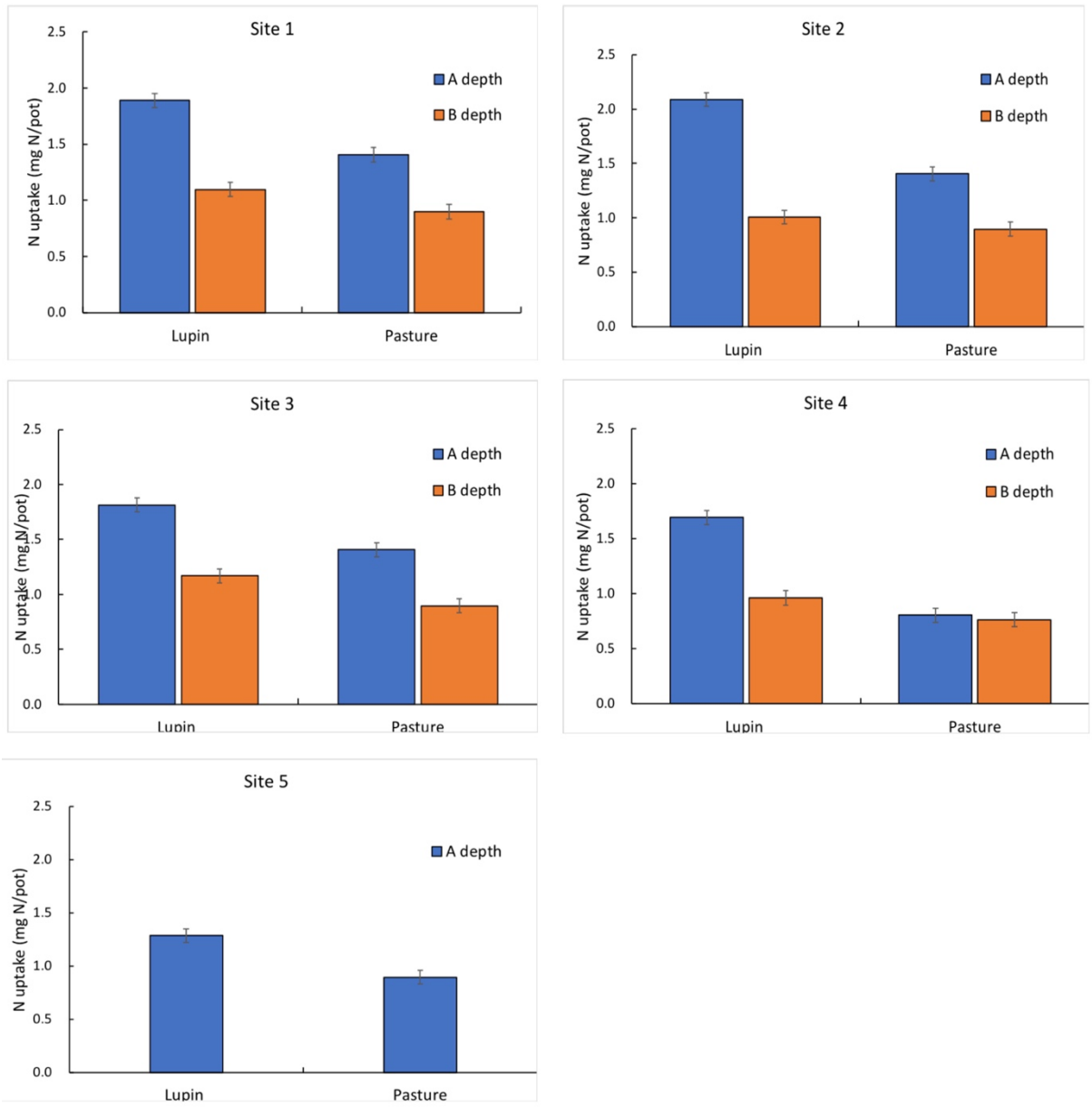

Figure 4.2: Nitrogen uptake by annual ryegrass grown in a glasshouse in soil collected from two depths in a perennial lupin stand and an adjacent pasture at each of five high country sites in the South Island. Lines on bars represent one SEM. Soil depth 'A' = 0-7.5 cm horizon, soil depth 'B' = 7.5-15 cm horizon.

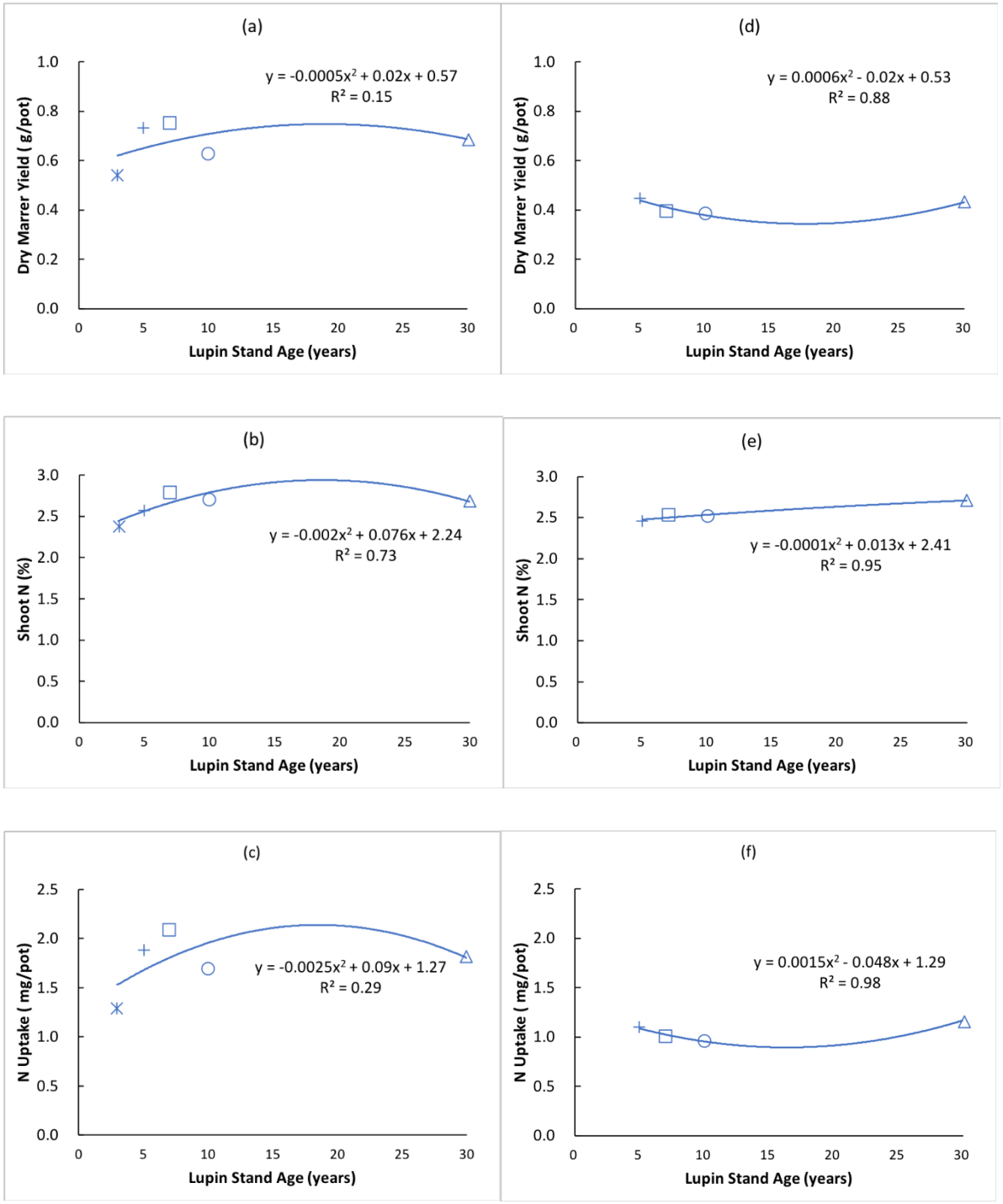

Figure 4.3: The relationship between mean dry matter yield, shoot N and N uptake concentration of annual ryegrass grown in a glasshouse in soil collected and analysed from two depths from perennial lupin stands of different age at each of five high country sites in the South Island. A depth = 0-7.5 cm (a,b,c) and B depth = 7.5-15 cm (d,e,f). Site 1: (+), site 2: (□), site 3:(△), site 4:(○), site 5:(∗).

4.4 Discussion

In the field study, soil N status in the soils of perennial lupin stands and long-term established pasture from the eight sites has been tested. It was demonstrated that lupin soils had higher soil labile N content than long-term established pasture soils. To more accurately quantify soil labile N status of the soils from the five sites, the glasshouse study was conducted, using annual ryegrass to take up the soil N and calculating soil N by plant dry matter yield and shoot N. Compared with the field study, the same result was shown in glasshouse study that soils in stands of perennial lupin showed higher soil mineralizable N than long-term established pasture soils for both soil horizon depths.

4.4.1 Temporal relationship between the N uptake and soil N level

The soils from five sites with various climate conditions, fertiliser application resulted in large differences between the soils from the five field sites. Ryegrass N uptake should be discussed in combination with ryegrass dry matter yield (DMY) and shoot N concentration. Site 1 and site 2 was Glenmore Station and Glenmore Station Original, respectively, with low to medium fertiliser application histories in the past (Table 3.1). The higher DMY, shoot N and N uptake values occurred at site 2, followed by site 1. Compared with the site 1 and 2, site 2 was Mount John Station, with a low fertiliser application rate which resulted in higher DMY, shoot N and N uptake than site 4 and site 5 with no fertiliser application. Fertiliser application increased soil N level increased soil fertility and increased mean ryegrass DMY and mean N uptake content from soils (Jarvis *et al*., 1996). Soil analysis also indicated the higher N content of site 2, which led higher N uptake by ryegrass (Table 4.2; Table 4.3). The significant effect also occurred with soil depths. Increasing soil depth decreased soil mineral and mineralizable N and decreased N uptake by ryegrass that Jobbágy and Jackson (2001) explained the results with more plant residue input to the topsoil. The N uptake content also significantly different between the forage types, which also showed close correlation to soil labile N levels. The higher soil N accumulation by perennial lupin lead to the higher soil mineral and mineralizable N resulted in higher N uptake by ryegrass. Figure 4.4 (a) showed the values of N uptake by ryegrass in glasshouse increased by increasing soil mineralizable N concentration. Did the values of soil N uptake by ryegrass and soil mineralizable N in the field study can show similar trend as the glasshouse study? For the same soil, changing the soil mineralizable N values from the glasshouse experiment instead by soil mineralizable N values in the field study. N uptake in the glasshouse experiment also related well to soil mineralizable N measured in the field experiment and showed a similar trend (Figure 4.4b).

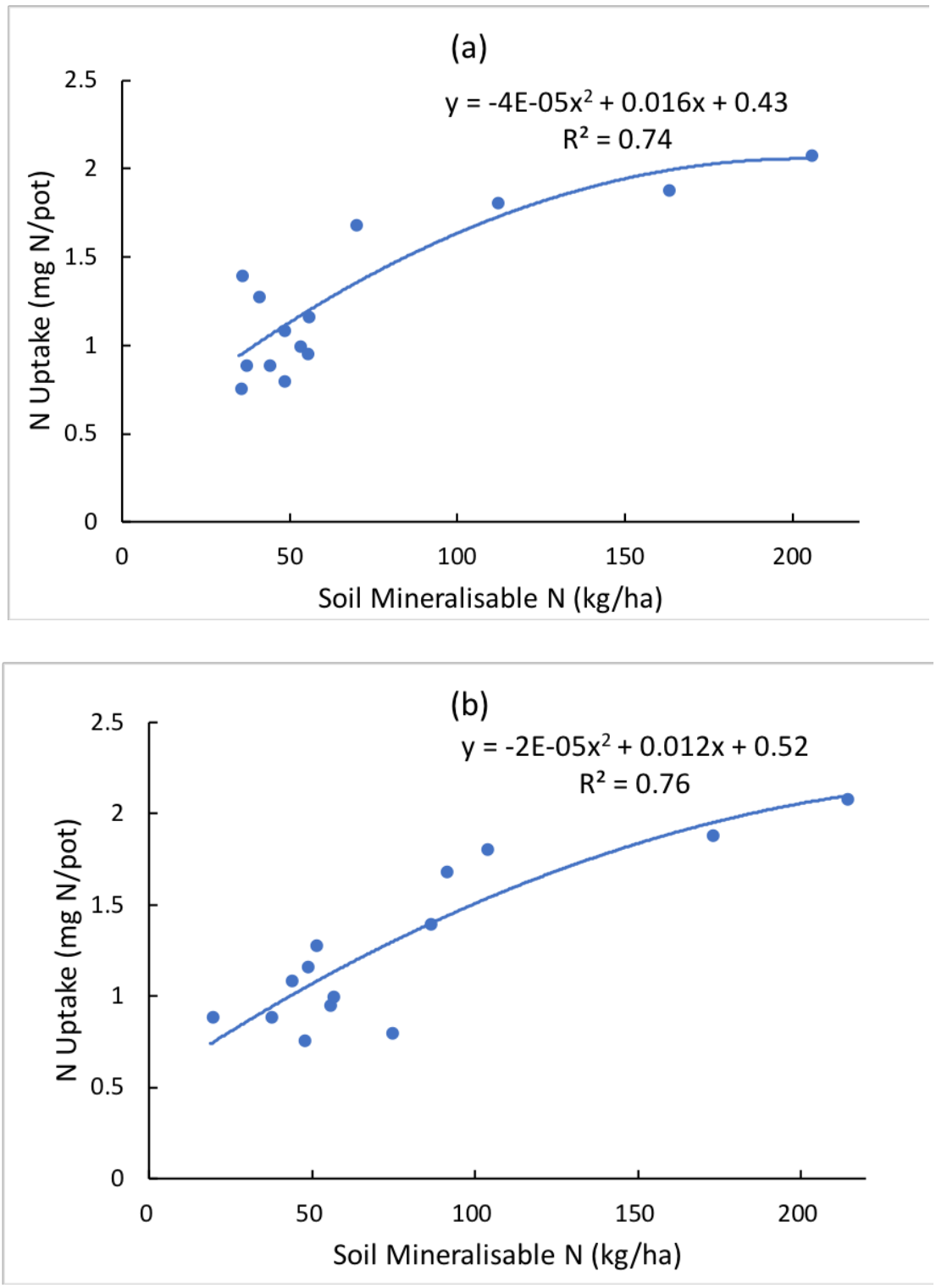

Figure 4.4: Mean N uptake per pot from glasshouse by the mean soil mineralizable N concentration collected from each site. The glasshouse soil mineralizable N was presented in (a) and field study soil mineralizable N was presented in (b).

4.4.2 Temporal relationship between the age of lupin stand and N uptake

For lupin soils only, ryegrass DMY and N uptake per pot showed significant ($P<0.001$) influence by lupin stand ages (Table 4.4). It should be discussed by two group. For sites 1, 2 and 5, mean ryegrass DMY, shoot N concentration and N uptake increased with increasing age of the lupin stands. Also, the increasing lupin stand ages increased mean DMY and N uptake by ryegrass also in site 3 and 4.

In the first group, sites 1, 2 and 5, there were several reasons which can explain the results. On the one hand, soil N accumulation increased by legume N fixation which increased with legume stand age. On the other hand, soil fertiliser application history resulted in higher ryegrass DMY and N uptake by ryegrass in site 1 and site 2 than site 5 (Table 3.1). The stony soil condition in Omarama Station may have limited the root development of lupin, which therefore may limit the N fixation by lupin and the N accumulation in the soil.

In the second group, sites 3 and 4, although the values of mean ryegrass DMY and N uptake were not higher than the first group (sites 1 and 2), soil N uptake by ryegrass also increased with increasing lupin stand ages. Jackman (1964) showed similar changes of soil N accumulation in response to the age of legume pasture. The lower values for sites 3 and 4, compared with soils from younger stands of lupin stand at sites 1 and 2, may have resulted from the low and nil fertiliser application in the past. The decreasing number of rhizobia and effectiveness may have also decreased soil N content for a long-term lupin stand, thus decreased ryegrass DMY and N uptake in glasshouse (Ballard *et al*., 2003).

Mean ryegrass DMY and N uptake was also significantly affected by soil depth with higher return of that highly plant residues in the topsoil increasing the soil N content of A depth and increased ryegrass growth in glasshouse and N uptake from soils (Jenny, 1941).

4.4.3 Soil mineralizable N and mineral N

The differences in soil mineral N and soil mineralizable N across the five sites were expected in this study. The different climates at the sites (Figure 3.5 and Table 3.1) resulted in various soil nutrient conditions. Soil mineralizable N was significantly affected by soil depths. Plant residues and biological cycling resulted in higher nutrients upwards (Stark, 1994). Thus, soil mineral N and mineralizable N showed higher values with A depth than B depth generally. However, the pasture soils showed higher values of soil mineral N with B depth than A depth which may cause by the highly variable nature of soil mineral N (Jarvis *et al*., 1996). Forage type also significantly influenced soil mineral N and mineralizable N. Soils from the lupin stands showed high values due to the N accumulation by legumes. In contrast, the lower N accumulation in pasture without legumes resulted in lower values than the soils from the lupin stands.

In this glasshouse experiment, there was the limitation of data by only five site soils involved. More soils from different lupin stands and long-term established pasture should be studied to understand the soil N status better and also better know about the changes of soil N status by lupin stand ages.

4.4.4 Dry matter yield

The highly significant differences of ryegrass dry matter yield between the soils from the five sites were also expected in this study. The five sites had various climate conditions which result in the variation in rainfall and temperature and therefore different soil conditions (Figure 3.5 and Table 3.3) (Brougham, 1959). The higher DMY for the soil from lupin stands resulted from generally higher soil N content in lupin stand soils (Table 4.1) (Novoa & Loomis, 1981). The highly significant difference of DMY in depths caused by various soil nutrient content in different soil depths. Jobbágy and Jackson (2001) reported that plant nutrient distributed shallower and decreased with depth by plant cycling. The litterfall on the soil surface increased the soil nutrient content in topsoil which led to the higher DMY of A depth than B depth in this study.

4.4.5 Shoot N

The different soil conditions across the five sites resulted in the highly significant influence on ryegrass shoot N level. Shoot N concentration was affected by, soil moisture, and nutrient availability (Smith & Loneragan, 1986). In the glasshouse experiment, the conditions of soil moisture and the plant species (annual ryegrass) were the same, while soil N level was varied across the five sites. The significantly different soil mineral N and soil mineralizable N for the five sites resulted in the variation of ryegrass DMY. Ryegrass shoot N concentration also significantly affected by soil depth that higher values of shoot N occurred in A depth. Increasing soil depth decreased soil N content and decreased ryegrass shoot N concentrates. Although, it has been reported that N improved the plant root growth and may also improve other nutrients uptake, the mechanisms of the process were not well understood (Adams, 1980; Wilkinson *et al*., 2000). Finally, the highly significant differences of shoot N between the soil from the lupin stands and the soil from the pastures could be explained by higher N content accumulated by lupin.

4.5 Conclusions

In this glasshouse experiment, the objective was to determine the effects of perennial lupin stands of different ages on the N concentration of acid high country soils. The glasshouse provided a controlled plant growth environment and decreased the effect of climate conditions and light conditions compared with field study of soil N.

The climate of various sites, different fertiliser application rates and rhizobia effectiveness were likely result in significant differences between soils from the five sites. Site 2 (Glenmore Station Original) showed highest values of soil mineralizable N and site 5 (Omarama Station) showed lowest soil mineralizable N value. Mean soil mineral N and mineralizable N significantly influenced by soil depth that A depth had higher values than B depth due to the higher plant residues accumulation and plant cycling on the topsoil. The highly significant difference in soil mineral N and soil mineralizable N also affected by forage types, where the lupin stand had higher values than pasture soils resulted from more N fixation by legume and accumulation in soil.

There was a close correlation between soil mineralizable N level and N uptake by ryegrass. Mean ryegrass DMY and N uptake per pot in glasshouse showed highly significant difference across the five sites. The increasing N content in soil led to higher ryegrass DMY and N uptake, and was also affected by soil depth and forage type with the same trend. Different sites, two depth and two forage types affected mean shoot N level as well, ranging from 1.91 to 2.79%

The ryegrass DMY and N uptake were related to the age of the lupin stands across the five sites. Generally, increasing lupin stand ages led to higher values in ryegrass DMY and N uptake. Site 3 and 4 with older lupin stands showed lower values than site 1 and site 2, which may cause by low fertiliser application in the past. However, the higher values of ryegrass DMY and N uptake also showed on the site with older lupin stand between site 3 and site 4. Soil depth showed a strong significant difference in ryegrass DMY and N uptake. Additionally, mean shoot N also significantly affected by soil depth. Increasing soil depth declined mean ryegrass DMY, shoot N and N uptake per pot.

Chapter 5

General Discussion

In these field and glasshouse studies, the forage type, age and climate conditions and fertiliser application in the past led to the highly significant differences in soil N content between different sites, included total soil N, soil mineral N, soil mineralizable N and N uptake by ryegrass. It has been reported that the soil moisture and temperature significantly affect soil N accumulation (Cassman & Munns, 1980; Hoglund *et al*., 1979). High annual rainfall with high temperature increased plant growth and resulted in higher N accumulation in the soil. Soil sampling for the field study was on 12 June 2017, and soil sampling for glasshouse experiment was on 8 November 2017. The soil analysis of soil N content showed the similar trend in field study and glasshouse experiment and the values in field study generally showed little higher than glasshouse experiment. The soil from the Glenmore Station Original site had the highest N content followed by the soil at the Glenmore Station site, and the soil from Omarama Station had lower N content in both studies.

There was a strong difference in soil N levels between lupin stands and adjacent long-term pasture sites. This showed that the perennial lupin resulted in higher N levels of total soil N, soil mineral N and mineralizable N than pasture soils in the field study, and also resulted in higher values in ryegrass DMY and N uptake in glasshouse study. Both studies showed higher N accumulation by lupin, potentially N fixation from atmosphere and released to the soil. Compared with pasture soils, lupin stand soils showed the significant increase of soil N content potentially due to the N fixation by legume and transferred to the soil in the field study. In addition, the lupin stands soils provided more N than pasture soils for ryegrass growth in the glasshouse experiment.

It has been reported that the N content increased by long-term pasture N accumulation with legume stand (Jackman, 1964). In lupin stand soils, total soil N and soil mineralizable N showed strong effects of lupin stand ages, which the site with relative older lupin stand age increased soil N level in the field study with site 1, 2, 3, and 8. The highest values of soil mineralizable N, 213.8 kg/ha occurred with the oldest lupin stand age, 7 years at the Glenmore Station Original site.

Compared with site 3, site 6 indicated much lower soil N content, which may have been caused by the high fertiliser application rate of site 3 and the stony soil condition of Omarama Station and lower annual rainfall at Omarama, limited root development of lupin. Site 7 and site 8 had the same lupin stand age showed large differences between soil N content, which might due to the higher rainfall in site 7, increased soil moisture for lupin growth and soil N accumulation and the low plant density of site 8, decreased N fixation.

In site 4 and site 5, soil N content indicated increased with lupin stand ages as well. However, the values of soil N content were lower than relative younger lupin stand soils for site 1 and 2. The reason of soil N level of such long-term lupin stand age possibly resulted from the interaction of climate, fertiliser application history, number of rhizobia and rhizobia effectiveness.

The same trend of soil N content was demonstrated in glasshouse experiment, where ryegrass DMY and N uptake by ryegrass indicated the soil N level indirectly. The long-term lupin stand soils (site 3 and 4) indicated higher soil N uptake by ryegrass with increasing lupin stand ages, which was also lower than the site with relative younger lupin stand (site 1 and 2). Soil N uptake by ryegrass with site 1, 2 and 3 increased with lupin stand ages.

For the lupin soils, there were significant differences between soil depth for total soil N and C, soil mineral N and soil mineralizable N in field study and the effect on ryegrass dry matter yield, shoot N and N uptake by ryegrass per pot in glasshouse study. Higher plant residues accumulation and transferred more soil N to the topsoil in the surface depth of soil.

Figure 4.4 indicated a close relationship between N uptake by ryegrass and soil mineralizable N from field study (a) and glasshouse study (b). Increasing soil mineralizable N increased N uptake by ryegrass. Higher soil mineralizable N content provided the higher potential of soil mineralization to increase soil mineral N plant uptake (Jarvis *et al*., 1996).

Soil depth significantly influenced total soil N and C, soil mineral N and soil mineralizable N in the field study. Without the climate distribution by natural environment, soil depth also influenced the values of soil N uptake by ryegrass in the glasshouse experiment. It indicated that soil N level strongly influenced by soil depth, where the higher values in A depth than B depth. Many studies showed the similar trend to the soil depth influence (Arrouays & Pelissier, 1994; Bernoux *et al*., 1998; Hossain *et al*., 1996; Jackman, 1964; Jobbágy & Jackson, 2001), that the higher nutrient accumulation in topsoil due to the plant residues.

Weathering, leaching, and biological cycling are the four main processes of the mechanisms of soil nutrient vertical distribution (Trudgill, 1988). Weathering and atmospheric deposition influenced the depth of nutrient inputs occurred (Kirkby, 1985). Leaching and biological cycling affected vertically transport of nutrients in opposite ways compared with weathering and atmospheric deposition. Leaching resulted in nutrients moving downward through the soil profile and increased nutrient concentration by increasing soil depth. Under field condition, soil N leaching may occur. The glasshouse experiment controlled the amount of water application, avoiding nutrient leaching. However, the similar N level illustrated in both study, which caused by critical influence from plant biological cycling. The nutrient cycling moved nutrient upwards for plant absorbed and then

transported back to the soil surface by falling of plant litters (Trudgill, 1988). Therefore, the main increase of soil N content with A depth resulted from plant residues accumulation.

Chapter 6

Conclusions and Future Research

6.1 Conclusions

For New Zealand high country soils, soil acidity limits the development of pasture and legume survival due to high exchangeable Al concentration and low soil fertility. Perennial lupin showed high adaptable for surviving in the New Zealand high country soils with low soil pH. To determine the soil the impact of perennial lupin stand ages on N concentration under acid high country soils, complementary field study and the glasshouse study were experimented.

Two hypotheses were tested in two experiments. Firstly, perennial lupin increased soil total and mineralizable N in plant rooting zone of high country soils. The higher soil N level occurred in lupin stand soils compared with pasture soils without legumes. The highest total soil N and soil mineralizable N values occurred for the Glenmore Station Original site with 0.68% and 213.8 kg/ha respectively, followed by Glenmore Station, Dasher Station and Mount John Station. The lowest value of total soil N and soil mineralizable N occurred at Lincoln University Campus with 0.21% and 36.85 kg/ha with the lupin-cocksfoot stand. The similar trend in glasshouse experiment on soil analysis result and ryegrass DMY and N uptake content also proved the higher N accumulation in lupin stand soils.

Secondly, the extent of soil N accumulation in growing lupin soils was dependent on lupin stand age. There was a highly significant relationship between soil N content and lupin stand age, which increasing lupin stand ages increased soil N accumulation in the soil. The glasshouse experiment tested soil N content by ryegrass growth. Generally, older lupin stands led to higher ryegrass DMY and N uptake from the soil. The highest N uptake value occurred for the soil from the Glenmore Station Original site as well, with 2.09 mg N/pot. The Mount John Station, with the oldest lupin stand of 30 years, did not have highest values of soil N content and N uptake by ryegrass, which probably caused by the interaction of relative older lupin stand age, fertiliser application history, climate and rhizobia level.

Soil depth had an effect on total soil C and N, soil mineral N, soil mineralizable N, ryegrass DMY, plant shoot N and N uptake by plant that increasing soil depth decline each value due to the plant residues accumulation and transfer nutrient to the soil surface.

Overall, only eight sites were studied in this research caused the limitation of the data. As such, it is important to confirm the findings reported here by further research that a large number of sites should be studied in the future.

6.2 Suggestions for further research

The effect of N accumulation by perennial lupin under different fertiliser application with various fertiliser types and application rates in New Zealand high country soils could be studied for knowing the potential of growing lupin for pasture farming.

It would be useful to find the way to reduce the concentration of alkaloid in lupin seed through genetic engineering. The mature seed with low alkaloid content would be easy to eat by ruminants. Low alkaloid lupin seed could provide more fodder to farming system.

Additionally, research about the extent of other soil nutrients such as micronutrient distributed in perennial lupin stand soils in New Zealand high country soils to provide the guidance for lupin and legume-based pasture production.

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